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# FORMATION OF PERIODIC PRECIPITATE IN THE ABSENCE OF A FOREIGN GEL., PART V. CERIC HYDROXIDE, CHROMIC HYDROXIDE AND CHROMIC ARSENATE SOLS.

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## SUMMARY

A study has been made of the periodic precipitation of ceric hydroxide, chromic hydroxide and chromic arsenate sols by the process of coagulation, in the absence of a foreign gel. The adsorption of sol by its own precipitate, the nature of the coagula which settle periodically and the speed of coagulation of the sol have been investigated to elucidate the phenomenon.

## Ceric Hydroxide Sol

The sol was prepared by subjecting a solution of ceric ammonium-nitrate to hot dialysis. The salt readily hydrolysed in water giving a sol of ceric hydroxide. The parchment paper bag in which the sol was dialysed was changed at intervals due to the digestive action of nitric acid, a product of hydrolysis, on the bag. After 3 hours of hot dialysis the sol was taken out for investigation. It contained 0·1199 g atom of Ce per litre and its purity was 7·05. It was diluted to 0·05995 g. atom of Ce per litre. The adsorption of sol, the volumes of the coagula and the speed of coagulation of the sol were determined as given in Part II of this series (Mittra, J. Indian Chem. Soc., 1939, **16,** 165).

TABLE I

Sol concentration 0·0995 g. atom of Ce per litre Sol taken each time 10 c.c. Total volume 20 c c. of which 9 c.c. contain 0·0002698 g. atom of Ce

| Amount of N/40 $K_2SO_4$ | Volume of coagulum | Amount of precipitate peptised |
|---|---|---|
| 5·5 c.c. | 0·25 c.c. | 0·000055 g. atom |
| 5·0 | 0·20 | 0·000084 |
| 4·5 | Partial coagulation | |

With this concentration and purity of the sol, no rings developed by coagulating with $K_2SO_4$, as the coagulum yielded was of lyophobic nature. Though the sol could be coagulated with saturated KCl, the preciptating concentration was not within range. Higher concentration of the sol behaved likewise.

Speed of coagulation

The coagulation of the sol with $K_2SO_4$ was almost instantaneous and hence the speed of coagulation of the sol could not be followed.

Adsorption of sol

TABLE II

Amount of precipitate taken each time 0·592 g. of Ce. Amount of sol taken each time 0·0825 g of Ce

| Amount of N/10-$K_2SO_4$ | Amount of Ce left in sol state | % Adsorption |
|---|---|---|
| 0 c.c. | 0·110 g. | −33·94 |
| 1 | 0·107 | −29·94 |
| 3 | 0·100 | −20·97 |
| 5 | 0·088 | − 6·6 |
| 7 | 0·066 | 20·54 |
| 9 | 0·000 | 100·00 |

In the absence of the precipitate, the same amount of the sol required 1·5 c.c. of the electrolyte for complete coagulation.

The original sol was further purified by hot dialysis for 15 hours. It contained 0·1359 g. atom of Ce per litre and its purity was 42·08. It was diluted to 0·05436 g. atom and 0·0408 g. atom of Ce per litre respectively.

TABLE III

Sol concentration 0·05436 g. atom of Ce per litre. Sol taken each time 10 c.c.
Total volume 20 c.c. of which 9 c.c. contain 0·000244 g. atom of Ce

| Amount of electrolyte | Volume of coagulum | Amount of precipitate peptised |
|---|---|---|
| N/2-KCl | | |
| 8 c.c. | 0·5 c.c. | 0·00010 g. atom |
| 7 | 0·5 | 0·00016 |
| 6 | Partial coagulation | |
| N/40-$K_2SO_4$ | | |
| 7 c.c. | 0·5 c.c. | Nothing |
| 6 | 0·5 c.c. | ,, |
| 5 | Partial coagulation | |

With this concentration and purity of the sol, quite prominent and broad rings developed, by coagulation with KCl (Plate I). The rings were separated by more or less clear spaces. The coagulation with $K_2SO_4$ was almost instantaneous with no developed rings.

TABLE IV

Sol concentration 0·0408 g. atom of Ce per litre. Sol taken each time 10 c.c.
Total volume 20 c.c. of which 9 c.c. contain 0·000184 g. atom of Ce

| Amount of electrolyte | Volume of coagulum | Amount of precipitate peptised |
|---|---|---|
| N/2-KCl | | |
| 8 c.c. | 0·45 c.c. | 0·000060 g. atom |
| 7 | 0·40 | 0·000098 |
| 6 | Partial coagulation | |
| N/40-$K_2SO_4$ | | |
| 4 c.c. | 0·45 c.c. | Nothing |
| 3 | 0·45 | ,, |
| 2 | Partial Coagulation | |

With this concentration of the sol, quite prominent rings had developed, by coagulation with KCl, but all were broken due to the complete settling of the coagula.

Speed of coagulation

TABLE V

6·8 c.c. of N/2-KCl made upto 10 c.c. coagulated 10 c.c. of the sol in 1 hour (Precipitating concentration)

| Time | Amount left in sol state out of 0·000295 g. atom of Ce |
|---|---|
| After 2 min. | No coagulation |
| 15 | 0·000284 g. atom |
| 45 | 0·000200 |
| 60 | 0·000180 |

Adsorption of sol

TABLE VI

Amount of precipitate taken each time 0·296 g. of Ce. Amount of sol taken each time 0·0936 g. of Ce

| Amount of N/2-KCl | Amount of Ce left in sol state | % Adsorption |
|---|---|---|
| 0 c.c. | 0·112 g. | −19·66 |
| 1 | 0·106 | −12·82 |
| 3 | 0·090 | 3·41 |
| 5 | 0·042 | 55·56 |
| 7 | 0·019 | 79·48 |
| 9 | 0·000 | 100·00 |

In the absence of the precipitate, the same amount of the sol required 20 c.c. of the electrolyte to start the coagulation.

The original sol was further purified by hot dialysis for 15 hours. It contained 0·1603 g. atom of Ce per litre and its purity was 100·21. It was diluted to 0·04809 g. atom of Ce per litre.

TABLE VII

Sol concentration 0·04809 g. atom of Ce per litre Sol taken each time 10 c.c.
Total volume 20 c.c. of which 9 c.c. contain 0·000266 g. atom of Ce

| Amount of electrolyte | Volume of coagulum | Amount of precipitate peptised |
|---|---|---|
| N/2-KCl | | |
| 5 − 2 c.c. | 0·6 c.c. | Nothing |
| 1 | Partial coagulation | |
| N/40 $K_2SO_4$ | | |
| 4 − 2 c.c. | 0·5 c.c. | Nothing |

With this concentration and purity of the sol, the coagula obtained by coagulating with KCl, were in gel state and no settling occurred even on long standing. The coagulation with $K_2SO_4$ was almost instantaneous. With lower concentration of the sol rings developed but due to complete settling of the coagula all were broken.

Speed of coagulation

TABLE VIII

2 c.c. of N/2-KCl made upto 10 c.c. coagulated 10 c.c. of the sol in 1 hour (precipitating concentration)

| Time | Amount left in sol state out of 0·000295 gm. atom of Ce |
|---|---|
| After 2 min. | 0·000290 g. atom |
| 15 | 0·000245 |
| 45 | 0·000109 |
| 60 | 0·000000 |

Adsorption of sol

TABLE IX

Amount of precipitate taken each time 0·296 g of Ce. Amount of sol taken each time 0·1104 g. of Ce

| Amount of N/4-KCl | Amount of Ce left in sol state | % Adsorption |
|---|---|---|
| 0 c.c. | 0·1232 g. | −11·60 |
| 1 | 0·1072 | 2·90 |
| 3 | 0·0832 | 24·64 |
| 5 | 0·0512 | 53·62 |
| 7 | 0·0000 | 100·00 |

In the absence of the precipitate, the same amount of the sol required 10·5 c.c. of the electrolyte for partial and 13·5 c.c. for complete coagulation.

CHROMIC HYDROXIDE SOL

The sol was prepared by adding ammonium hydroxide to chromic chloride short of precipitation, until the precipitate of chromic hydroxide was peptised with difficulty. The resulting sol was dialysed hot for 30 hours. It contained 0·3368 g. atom of Cr per litre and its purity was 2·26. It was diluted to 0·1684 g atom of Ce per litre.

TABLE X

Sol concentration 0·01684 g. atom of Cr per litre. Sol taken each time 10 c.c. Total volume 20 c.c. of which 9 c c. contain 0·000076 g.atom of Cr

| Amount of N/40 $K_2SO_4$ | Volume of coagulum | Amount of precipitate peptised |
|---|---|---|
| 3·5 c.c. | 0·6 c.c. | Nothing |
| 3·0 | 0·5 | ,, |
| 2·5 | Partial coagulation. | |

With this concentration of the sol, very few rings, not so well defined, appeared with 3·0 c.c. of the electrolyte. Higher amounts of the electrolyte coagulated the sol instantaneously. Higher concentrations of the sol yielded coagula in gel state with no settling.

Speed of coagulation

TABLE XI

2·5 c.c. of N/10$K_2SO_4$ coagulated 10 c.c. of the sol in 1 hour (precipitating concentration)

| Time | Amount left in sol state out of 0·000295 g. atom of Cr |
|---|---|
| After 2 min. | 0·000162 g. atom |
| 15 | 0·000084 |
| 45 | 0·000084 |

Adsorption of sol

TABLE XII

Amount of precipitate taken each time 0·27 g. of Cr. Amount of sol taken each time 0·048 g. of Cr

| Amount of N/40$K_2SO_4$ | Amount of Cr left in sol state | % Adsorption |
|---|---|---|
| 0 c c. | 0·057 g. | − 18·75 |
| 1 | 0·054 | − 11·25 |
| 3 | 0·038 | 20·83 |
| 5 | 0·000 | 100·00 |

In the absence of the precipitate, the same amount of the sol required 15 c.c. of the electrolyte to start the coagulation.

The original sol was further purified by hot dialysis for 30 hours. It contained 0·31 g. atom of Cr per litre and its purity was 3·6. It was diluted to 0·0155 g. atom of Cr per litre.

TABLE XIII

Sol concentration 0·0155 g. atom of Cr per litre. Sol taken each time 10 c.c. Total volume 20 c.c. of which 9 c.c. contain 0·000069 g. atom of Cr

| Amount of electrolyte 2N-KCl | Volume of coagulum | Amount of precipitate peptised |
|---|---|---|
| 2 c.c. to 3 c.c. | 0·4 c.c. | Nothing |
| 1·5 c.c. $N/100K_2SO_4$ | Partial coagulation | |
| 3·5 c.c. to 4 c.c. | 0·4 c.c | Nothing |
| 3·0 c.c. | Partial coagulation | |

With this concentration of the sol, no prominent rings developed with KCl, though slow coagulation occurred. The coagulum in gel state, however got separated in several portions followed by clear spaces, forming gel bands (Plate II) $K_2SO_4$ coagulated the sol instantaneously with no rings developed.

Speed of coagulation

TABLE XIV

2·5 c.c. of 2N-KCl (made up to 10 c.c.) coagulated 10 c.c. of the sol in 1 hour (precipitating concentration)

| Time | Amount left in sol state out of 0·000295 g. atom of Cr |
|---|---|
| After 2 min. | No coagulation |
| 15 | 0·000185 g. atom |
| 45 | 0·000078 |
| 60 | Nothing |

Adsorption of sol

Amount of precipitate taken each time 0·27 g. of Cr. Amount of sol taken each time 0·085 g. of Cr

Complete adsorption took place in the absence of electrolyte.

CHROMIC ARSENATE SOL

The sol was prepared by adding a solution of acid potassium arsenate to chromic chloride solution and the resulting sol was subjected to cold dialysis for 5 days. It contained 0·3492 g. atom of Cr per litre and its purity was 1·80. It was diluted to 0·0175 g. atom of Cr per litre.

TABLE XV

Sol concentration 0·0175 g. atom of Cr per litre. Sol taken each time 10 c.c. Total volume 20 c.c. of which 9 c.c. contain 0·0000787 g. atom of Cr

| Amount of $N/40K_2SO_4$ | Volume of coagulum | Amount of precipitate peptised |
|---|---|---|
| 2·0 c.c. | 0·45 c.c. | Nothing |
| 1·5 | 0·50 | ,, |
| 1·0 | Partial coagulation | |

With this concentration of the sol, a few incomplete rings appeared (Plate III). The coagulation with this electrolyte was almost instantaneous. Higher concentrations of the sol yielded coagula in gel state with no settling.

Speed of coagulation

TABLE XVI

4·5 c.c. of N/40$K_2SO_4$ (made up to 10 c.c.) coagulated 10 c.c. of the sol almost instantaneously (precipitating concentration)

| Time | Amount left in sol state out of 0·000295 g. atom of Cr |
|---|---|
| After 2 min. | 0·000093 g. atom |
| „ 15 „ | 0·000043 |
| 30 | 0·000 |

The original sol was further purified by cold dialysis for 15 days. It contained 0·3589 g. atom of Cr per litre and its purity was 7·77. It was diluted to several extents and the compact volumes of the coagula were noted by coagulating with 2N-KCl and N/40 $K_2SO_4$. In each of the cases it was seen that the coagula developed so stiff gels that no settling occurred even on long standing.

There occurred complete adsorption of sol by its own precipitate in the absence of electrolytes.

From the foregoing results it may be seen that periodic precipitation of ceric hydroxide sol is most favourable when the coagulum, obtained from 9 c.c of the total volume of 20 c.c. of the sol-electrolyte mixture, has an optimum volume of 0·5 c.c. on centrifuging. Along with it the speed of coagulation must be slow so as to give a S-shaped character to the coagulation-velocity curve (table V). No rings are obtained with $K_2SO_4$ as coagulant as the coagulation with this electrolyte is instantaneous, with whatever purity of the sol tried. In the case of chromic hydroxide sol of purity 2·26 the coagulation with $K_2SO_4$ is not instantaneous (table XI) and there is tendency for the formation of rings, but the speed of coagulation is not slow enough to give rise to the formation of prominent rings. The condition is still more unfavourable with chromic arsenate sol of purity 1·8 When both these chromic hydroxide and chromic arsenate sols are purified to be just coagulated with KCl which produces slow coagulation, the coagula from such sols get so much hydrated that they do not settle at all even on long standing.

The data on the adsorption of sol show that as the sols are purified the adsorption increases with least facility for the formation of rings.

PLATE I

R. N. MITTRA—*Ceric Hydroxide Sol.*

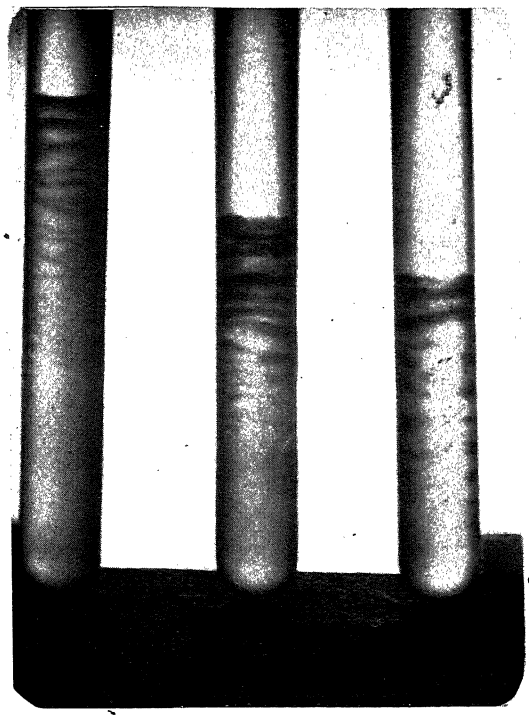
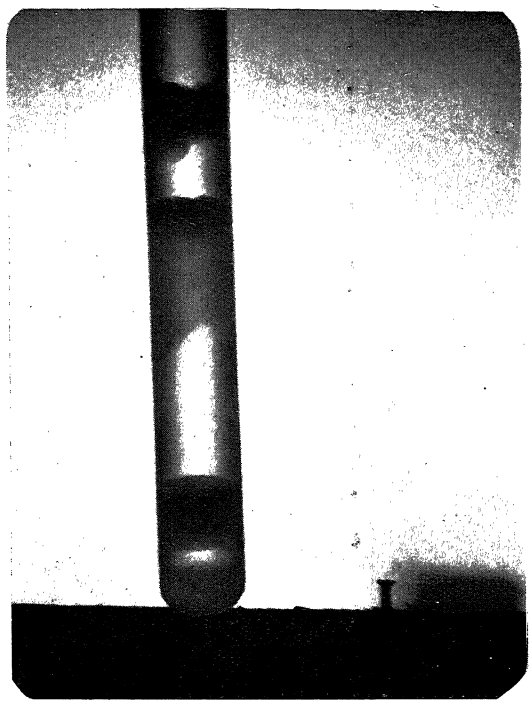


PLATE II

R. N. MITTRA—*Chromic Hydroxide Sol.*



# FORMATION OF PERIODIC PRECIPITATE IN THE ABSENCE OF A FOREIGN GEL, PART VI

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SUMMARY

This paper gives a general condition of the coagula, which can settle periodically, obtained from all the sols studied in this series and discussions on the results obtained.

In previous publications of this series (Mittra, Proc Nat. Acad. Sci., 1936, **6**, 332; 1939, **9**, 131, 138; J. Indian Chem. Soc., 1939, **16**, 165; this issue) it has been reported for the first time by the author that the sols of ferric hydroxide (prepared by acetate and carbonate methods), ferric phosphate, ferric arsenate, ferric borate, ceric hydroxide, chromic hydroxide, chromic arsenate, give rise to periodicity in their coagula when they are subjected to slow coagulation by the addition of mono or bivalent electrolytes according to their respective nature and purity. The formation of periodic precipitates of insoluble substances by the interaction of two solutes in gel medium recalls the investigation of Liesegang in 1896. Large amounts of similar observations were made in recent years by several workers (Bradford, Bio. Chemical Journal, 1921, **15**, 554; 1916, **10**, 169; Science Progress, 1916, **10**, 369. Dhar and Chatterji, Kolloid Z., 1922, **31**, 15; 1925, **37**, 2; 1926, **40**, 97. Hedges and Henley, Jour. Chem. Soc., 1928, 2714). Periodicities in sparingly soluble substances without the presence of a gel have also been reported by some workers, (Fischer and Schmidt, Rocz. Chem , 1926, **6**, 404. Capisarow, Jour. Chem. Soc., 1927, 222. Morse, J. Phys. Chem., 1930, **34**, 1554) when a reacting solution diffuses into the other held in capillary spaces or tubes.

The formation of periodic precipitates investigated by the above workers depend primarily on the formation of a sparingly soluble substance obtained by a preceding chemical reaction. It should, clearly, be stated that in the production of rings by a general double decomposition method in a gel medium, there are three stages which guide the phenomenon: (1) formation of an insoluble substance by the chemical interaction between two reacting solutes, (2) aggregation of the insoluble substance to form colloidal aggregates, and finally (3) the precipitation of the colloidal material in a periodic manner. The first stage of the process has been considered as no less an important factor for the production of rings by Wo Ostwald (Kolloid Z., 1926, **40**, 144). He attributes the formation of periodic

structures to the chemical interaction occurring between two reacting solutes in a gel medium, in waves. The points of interference of such wave-like motions of the reacting agents and also of the third product of the reaction have been supposed to be the nodes where the insoluble substance gets precipitated giving rise to periodic structures.

With the simple process carried on by the author in obtaining the periodic precipitates, the first stage of the process has completely been eliminated. That is, no preceding chemical reaction is necessary for the production of the insoluble substance which already exists in the colloidal condition.

The sol of partially lyophilic nature are prepared at different stages of purity. 10 c.c. portion of these sols are mixed with different electrolytes in separate test tubes, the total volumes being made up to 20 c.c. The tubes are kept for 24 hours for slow coagulation. From the results on the sols studied in this series it has been shown that an optimum volume of the coagula is necessary for their settling periodically. When 9 c.c. of the suspension obtained on thorough shaking of the coagula which has settled periodically, out of the total volume of 20 c.c. of the sol-electrolyte mixture, are centrifuged for 5 minutes, at a revolution of 2000 r. p.m. the compact volume of the coagulum is 0·5 c c. But with increase in purity of the sol the coagulum gets more hydrous and does not settle in a periodic manner, inspite of maintaining the concentration of the sol such that the optimum volume is reached. Thus in order to make out a general condition of all the sols which can guide the formation of rings, a comparative study of the sols have been found necessary. Along with the study already made the sols were maintained at the same concentration of 0·07378g. atom of the metal, constituting the sol, per litre. 10 c.c. of the sol were coagulated by different concentrations of electrolytes and the total volumes were made up to 20 c.c. After an hour the content of the tube, in which coagulation has just occurred, was shaken thoroughly and 8 c.c.f rom it with a metal content of 0·000295g. atom were subjected to centrifuge at a speed of 2000 r. p. m. for 5 minutes. The compact volume of the coagulum was noted. The following are the results obtained with different sols studied in this series.

| Purity. | Volume of coagulum (with KCl). | Volume of coagulum (with $K_2SO_4$). |
|---|---|---|
| | Ferric hydroxide sol (acetate). | |
| 0·57 | 0·45 c.c. | ... |
| 2·06 | 1·0* | ... |
| 6·66 | 1·3 | ... |
| | Ferric hydroxide sol (carbonate). | |
| 1·12 | ... | 0·9 c.c. |
| 4·93 | 1·0* | ... |
| 8·62 | 1·3 | ... |

| | | |
|---|---|---|
| | Ferric hydroxide sol (Krecke's). | |
| 21·70 | 0·15 | ... |
| 120·60 | 0·25 | ... |
| | Ferric phosphate sol. | |
| 0·50 | ... | 1·0* |
| 1·14 | ... | 1·0 |
| 1·92 | ... | 1·2 |
| | Ferric borate sol. | |
| 1·24 | ... | 1·0* |
| 2·78 | ... | 1·2 |
| 5·62 | ... | 1·4 |
| | Ceric hydroxide sol. | |
| 7·05 | ... | 0·3 |
| 42·08 | 1·0* | ... |
| 100·21 | 1·3 | ... |
| | Chromic hydroxide sol. | |
| 2·26 | .. | 1·1 |
| 5·87 | 3·5 | 2·2 |
| | Chromic arsenate sol. | |
| 1·80 | ... | 1·4 |

From the above table it is seen that the sols are best suited for the production of rings the coagula from which containing 0·000295g. atom of the metal constituting the sol, occupy a compact volume of 1·0 c.c. Along with it the speed of coagulation either with mono or bivalent coagulating ions must be slow. It will be of interest to note here that in some cases bivalent coagulating ions produce slow coagulation but with very impure sols. It appears that the charge neutralisation by monovalent coagulating ions generally and by bivalent ions only in impure sols is a slow process and this is due to the presence of a large amount of the stablishing ions in the sols. It is probable that during such a process of slow coagulation, the electric charge on the colloid particles may not diminish to the extent of minimum potential. The adsorption of the electrically charged particles by the precipitated material obtained by the partial coagulation of the sol, may take down the whole of the dispersed material and the sol thus gets completely coagulated. This may also be seen from data on the rate of coagulation. The coagulation proceeds at first

* The asterisks *indicate the volume where best rings develop.

very slowly and then rises quickly and finally falls off towards the completion of the process The velocity-coagulation curves are therefore S-shaped. This autocatalytic nature of the curves can only be explained by the adsorption of the uncoagulated sol by the coagulum already appeared, and this adsorption increases enormously with more of the coagulum appearing. It has generally been observed that when the coagulum, obtained from a sol coagulated completely by a minimum quantity of electrolyte, is shaken and centrifuged it leaves some of it in sol state, and the amount of coagulum thus peptised is in most cases comparatively larger when the coagulation is effected by monovalent electrolytes. This evinces that some of the colloid particles are mechanically carried down by the partially coagulated material. In the case of purer sols, the charge neutralisation of the colloid particles is more or less complete. This leads to greater aggregation of the particles and very little or no precipitate is peptised while centrifuging. Thus adsorption plays an important role along with slow coagulation in the process of ring formation. When partially purified sol is subjected to slow coagulation, the coagula which appear in stages have the facility for co-existing with the uncoagulated sol and form adsorption centres where the first set of nuclei started. With time the adsorption centres get thickened up by adsorbing more sol and form uniform bands in the shape of rings This process of adsorption is further facilitated by the slight settling of the coagula. The rings are held up in position due to the hydrous condition of the medium.

If the purification of the sol be carried on too far, the range of slow coagulation shortens considerably and the coagulation velocity curve is not S-shaped but takes an asymptotic nature. The coagulum also gets more hydrous and its compact volume containing 0·000295 g. atom of the metal goes beyond 1·0 c.c. No rings are obtained at this stage though maximum adsorption takes place of the sol by its own precipitate This may also be seen from the experiments that the amount of precipitate getting peptised on centrifuging decreases considerably with increase in purity of the sol. That is, the loosely adsorbed sol by the precipitated mass gets more firmly adsorbed on purification. The adsorption experiments carried on during the study also show that the adsorption of sol by its own precipitate goes on increasing with increase in purity of the sol, where the facility for ring formation is the least. Thus adsorption of sol by its own precipitate is a general phenomenon in colloids and is not a criterion in the process of ring formation. Any sol will give rise to periodicity in its coagulum which satisfies the two factors: (1) the speed of coagulation and (2) the nature of coagulum.

In conclusion, the author wishes to express his indebtedness to the authorities of the University of Allahabad for granting him the D. Sc. Research Scholarship for the period the work was being carried on.

# PHYSIOLOGICAL STUDIES ON THE WHEAT PLANT. PART III THE CHLOROPHYLL AND CARBOHYDRATE CONTENTS OF *TRITICUM VULGARE* IN RELATION TO MANURES

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SUMMARY

The quantities of all the four pigments show wide fluctuations during the ontogenic drift of the Wheat plant. These fluctuations are noticed in plants growing in the Subsoil, Molasses, Compost and Control beds.

The monosaccharides are the highest in the young stage and rapidly decline off as the age of plants advanced. The disaccharides, however, show a maximum concentration when the life cycle of the plant is half completed

There is no definite correlation between the plant pigments and the soluble carbohydrates. This proves that the pigments under the given experimental conditions are above the limiting value.

## Introduction

In a previous paper on Wheat[10], from this laboratory, the investigation was undertaken with a view to establish a correlation, in any, with amino and total nitrogen and the yield.

In continuing the work on Wheat, in the present paper, we have attempted to correlate the carbohydrate and chlorophyll contents of plants in relation to different soils.

Willstätter and Stoll[15] in their discussion on the relation of the chlorophyll content to photosynthetic rate showed :—

(1) that chlorophyll does not change during photosynthesis,

(2) ratio of chlorophyll A and B and that of the yellow pigments remains nearly constant during photosynthesis,

(3) and that with an increase of chlorophyll content there is also an increase in photosynthesis. There is, however, not a direct proportionality between the two, and the two are by no means parallel.

So far as we know, no work of any importance has been done on the ratio of the formation of the pigments to the manurial content of the soil, also no definite correlation has been established between the salts of the soil, the chlorophyll and the carbohydrates of the leaves.

We have attempted here to show whether any correlation exists between soil factor, chlorophyll and the sugars of the leaves, and their final yield.

## Material and Method

*(a) Seeds.*—Pusa no. 52 strain was sown in the Botanical Gardens, University of Allahabad, by the broadcast method on October 15, 1938. A few seedlings were also germinated in the Sawdust. The first experiments on wheat were carried out when the plants were 6 to 11 days old.

*(b) Estimation of sugars.*—Sugars were determined by Benedict's[6] and Dastur and Samant's[4] methods.

For determining the sugars, about 2—5 gms. of leaves were taken and immediately killed by putting in boiling water and boiled for about 2—3 minutes. They were then crushed into a paste with a little sand in a hand mortar. A pinch of basic Lead Acetate was then added. The leaf extract was then decanted and filtered through a Suction filter and a slow current of sulphuretted hydrogen was passed through in order to precipitate completely the insoluble black Lead sulphide. The solution was filtered again. The volatile $H_2S$ was driven out and the solutions were then concentrated to a given volume. They were then divided into two parts, one of which was used for estimating the monosaccharides and the other for the disaccharides.

Dastur and Samant's[4] method of phosphomolybdic acid was also tried. They used two standard solutions.

(*a*) $CuSO_4$ solution containing $Na_2CO_3$ and tartaric acid is kept in the dark in a coloured bottle,

(*b*) Phosphomolybdic acid solution.

According to them 2 c.c. of solution "B" would render 2 c.c. of solution "A" colourless. We, however, failed to get such a result and found that even the addition of 4—5 c c of "B" to 2 c.c. of "A" would not completely render the solution colourless, a slight bluish-green tinge would remain persistent.

This blue colour was then matched with the blue colour of the standard solution with the Duboscq-Colorimeter after 40 minutes of the production of the blue colour.

*Standard Sugar Solution.*—consisted of 0·002% of anhydrous glucose solution, in which a little toluene was added.

We attempted the tartaric acid method of the hydrolysis of the disaccharides as suggested by Dastur and Samant, but found that hydrochloric acid gave more satisfactory results.

In Benedict's solution for the estimation of reducing sugars, the difficulty of the red precipitate of Cuprous Oxide, obscuring the end point is overcome by carrying out the reduction in the presence of KCNS, whereby the Cuprous Oxide is converted into an insoluble white compound and thus the disappearance of the last trace of blue colour from the solution is ready to observe.

1 c c. of Benedict's solution = 0·002 gms. of glucose.

As Benedict's solution gave us better results, in our later experiments we have followed this method,

(c) *The quantitative estimations of the pigments*:—The chlorophyll determinations were carried out by the help of the Hellige-Duboscq-Colorimeter with the use of the Inorganic standard solutions, given as follows according to Guthrie[5] :—

(1) 11·40 gms. of pure Copper sulphate dissolved in water and the solution made to one litre,

(2) 20 gms. of recrystallised $K_2Cr_2O_7$ dissolved in water and solution made to one litre,

(3) 2M solution of ammonia (mol. wt. 35·05) with the help of a hydrometer.

The colour standard was prepared by mixing 25 c.c. of $Cu_2SO_4$ soln. and 50 c.c. of $K_2Cr_2O_7$ and 10 c.c. of ammonia and making to 100 c.c. in a measuring flask.

*Standard solution for yellow pigments.*—2 gms. of recrystallised Potassium bichromate were dissolved in water and the solution made to one litre.

25 c.c. of this were made up-to one hundred c.c. for the standard.

*Procedure.*—1 gm. of fresh green leaf was accurately weighed and finely crushed with a little washed sand and washed with 35% acetone repeatedly over a Büchner funnel until the filtrate was colourless. This dirty brown acetone solution was rejected. The dry residue over the Büchner funnel was next treated with pure acetone repeatedly to extract all the chlorophyll pigments (until the filtrate was absolutely colourless). To this about 10 c c of water and ether-petroli about twice the volume of acetone was added in a separating funnel, and acetone washed with water several times to remove all the traces of acetone. The ethereal solution of the leaf pigments was then saponified with 30% KOH in methyl alcohol, in the cold, and two layers were formed, the upper one brilliant green containing the green pigments A and B, and the lower one transparent pale yellow containing Carotin and Xanthophyll. The green pigments brought to a volume of 25 c.c. were next matched with Guthrie's green, previously prepared, with the Hellige-Duboscq Colorimeter.

*Separation of Carotin and Xanthophyll.*—The ethereal solution of Carotin and Xanthophyll was washed carefully with distilled water and evaporated to dryness and then about 35 c.c. of petroleum ether and 15 c.c. of 85% methyl alcohol was added to it, in a separating funnel. The lower layer of Xanthophyll was run off in another separating funnel and freed from any Carotin by adding a few c.c. of petroleum ether. The upper layer contained Carotin. In some cases when the extract were cloudy a few drops only of absolute alcohol, were added to clear.

## Experimental Results

The examination of the chlorophylls were taken in the manner given above. From Fig. 1 it is evident that the variation of the quantities of chlorophyll at

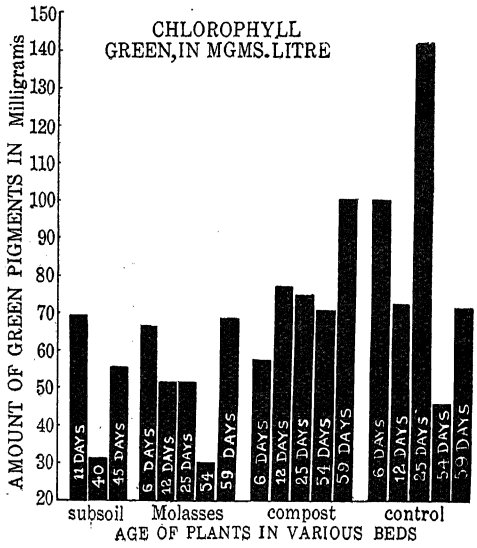


Fig. 1

different ages of the plants in the various soils does not follow any uniform principle, and therefore broad generalizations can only be made. Generally speaking, we may say that the chlorophyll of plants growing in the Subsoil and Molasses beds are less than the chlorophyll plants in the Compost and Control beds.

## Carotin and Xanthophyll

While it was difficult to deduce the chlorophyll content of plants in various beds to a given scheme, it has been more difficult to bring the Carotin and Xanthophyll concentrations of plants to any uniform system.

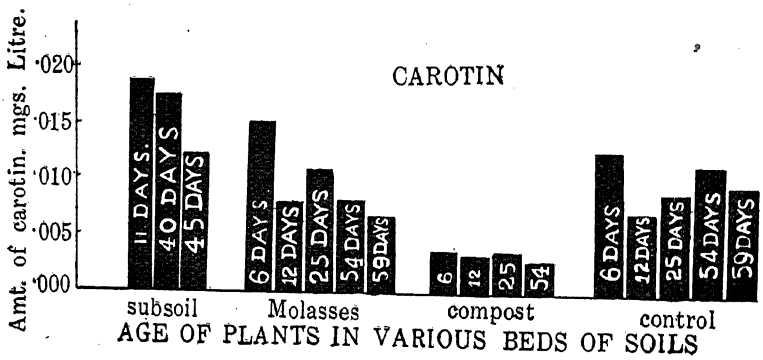


Fig. 2

Fig. 2 for Carotin shows that the amount of this pigment in plants growing in the Compost beds was small, while the contents of Carotin in the Control, Molasses and Subsoil seem to be pretty nearly the same.

Xanthophyll, on the other hand (Fig. 3), seems to be in general the same in plants growing in the various beds.

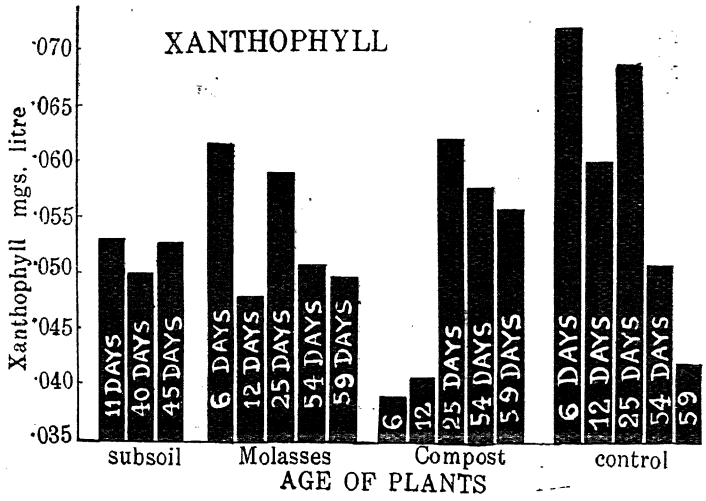


Fig. 3

Fig. 3 shows the development of Xanthophyll pigments in various soils.

*Monosaccharides and Disaccharides.*—Fig. 4 and 5 for the monosaccharides and the disaccharides are slightly more regular. In practically all cases, the values of the monosaccharides are very high for plants 6 days old. This value then rapidly falls and then keeps to a fluctuating low level.

In the case of the disaccharides (Fig. 5) the curves seem to be of a more or less binomial type. Here the values at both the start and end of experiments is each low, while invariably the highest peak is reached when the life cycle of the plant is half completed.

*Yield of grain and straw per seed sown.*—During the month of March, when the ear ripened, the plants growing from one seed sown were carefully removed by the randomisation method from each of the beds. All the ears were removed from the straw in each plant, they were put in a drying oven and dried at 95°C. and their dry weights then taken. The results are as follows:—

| Beds. | Grains in grams. | Straw in grams. |
|---|---|---|
| Compost ... ... | 1·825 | 2·409 |
| Control ... ... | 1·782 | 2·21 |
| Molasses ... ... | 1·612 | 1·98 |
| Subsoil ... ... | 1·320 | 1·177 |

## Discussion of the Results

*The relation of plant pigments to the soluble carbohydrate in the leaf.*—According to Willstätter and Stoll, the chlorophylls in certain cases influence the rate of

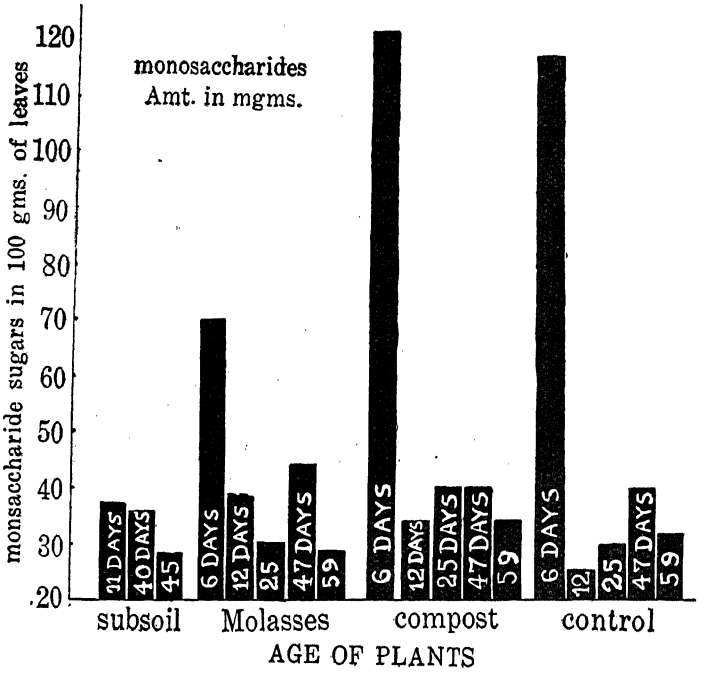


Fig. 4

photosynthesis. Our results here, strictly speaking, do not give us any very clear ideas on this subject for we could not take into account the other metabolites formed as a result of photosynthesis, except the mono and disaccharides. We confess, our data is rather meagre, but from what little evidence we have, no close correlation can be established. Taking the case of the monosaccharides, we get the highest value as is natural, in the very young stage, when the chlorophyll content was relatively low. The monosaccharides then fall without any relation to the chlorophylls present in the leaves. Then again the case of the disaccharides is so different from the monosaccharides and bears no relation with either the monosaccharides or the chlorophyll contents, see Figs. 1, 4, and 5.

The relation between the Hydrocarbon pigments and the soluble carbohydrates are also non-existent, for whereas in the case of Carotin, the pigment is the least in plants growing in the Compost bed, the quantity of both the mono and disaccharides is by no means small.

We are thus lead to believe that in all the beds (Subsoil, Molasses, Compost and Control) there was ample provision for the development of pigments in plants, and these pigments were above the limiting value under the given experimental conditions. Thus the slight variations of these pigments had no effect on the actual formation of the metabolites during photosynthesis.

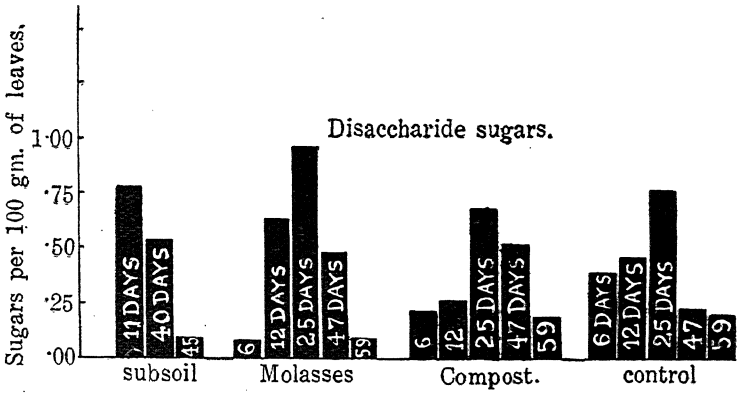


Fig. 5

*Total yield in relation to the various soils.*—Though no relation could be established between the pigments per given weight of plants and the soluble carbohydrates, yet it is abundantly clear that there has been wide differences in the yield of the crop in the various beds.

It was noticed that far more tillers appeared in the case of plants growing in the Compost than what was found in the Molasses or Subsoil beds. Thus the total reactive surface was far greater in the Compost bed which was thus responsible in giving greater yield of the total quantity of the grain. An attempt will be made in the next paper, following this, as to account for the causes responsible for the greater production of these as tillers, but we may safely conclude here, that so far as the chlorophyll content was concerned, it was present in adequate quantities to allow an optimum photosynthetic activity per given weight of the plant material.

APPENDIX 1

| Age of plants in days. | Plot. | In milligrams per litre. | | | In grams per 100 gm. of fresh leaves. | |
|---|---|---|---|---|---|---|
| | | Chlorophyll | Xantho-phyll | Carotin | Monosac-charides | Di-Sugars |
| 8 | Sawdust ... | 50·2 | 0·054 | 0·018 | 2·0 | 0·50 |
| 6 | Molasses ... | 67·0 | 0·062 | 0·0152 | 1·2 | 0·07 |
| | Compost ... | 57·0 | 0·039 | 0·0046 | 2·5 | 0·2 |
| | Control ... | 100·7 | 0·0724 | 0·0130 | 2·24 | 0·39 |
| 14 | Sawdust ... | 30·5 | 0·052 | 0·027 | 0·75 | 0·14 |
| 12 | Molasses ... | 51·9 | 0·048 | 0·008 | 0·47 | 0·63 |
| | Compost ... | 60·3 | 0·041 | 0·0036 | 0·36 | 0·29 |
| | Control ... | 72·6 | 0·0615 | 0·0078 | 0·14 | 0·46 |
| 25 | Molasses ... | 51·9 | 0·059 | 0·011 | 0·20 | 0·90 |
| | Compost ... | 77·7 | 0·062 | 0·0041 | 0·50 | 0·68 |
| | Control ... | 144·0 | 0·069 | 0·009 | 0·25 | 0·78 |
| 11 | Subsoil ... | 69·0 | 0·0537 | 0·0189 | 0·44 | 0·79 |
| 47 | Molasses ... | ... | ... | ... | 0·60 | 0·47 |
| | Compost ... | ... | ... | ... | 0·51 | 0·56 |
| | Control ... | ... | ... | ... | 0·47 | 0·255 |
| 54 | Molasses ... | 30·0 | 0·051 | 0·0082 | ... | ... |
| | Compost ... | 75·4 | 0·058 | 0·003 | ... | ... |
| | Control ... | 44·7 | 0·051 | 0·012 | ... | ... |
| 40 | Subsoil ... | 32·2 | 0·049 | 0·0174 | 0 39 | 0·56 |
| 59 | Molasses ... | 69· | 0·047 | 0·007 | 0·21 (V. few ears were developed). | 0·05 |
| | Compost ... | 70·8 | 0·056 | 0·0032 | 0·36 in Leaf; 2·06 in ears | 0·18 in Leaf; 0·94 in ears |
| | Control ... | 72·0 | 0·042 | 0·0107 | 0·317 (Leaf) 1·87 (ears) | 0·2 (Leaf) 0·87 (ears) |
| 45 | Subsoil ... | 55·0 | 0·053 | 0·0137 | 0·20 | 0·07 |

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# A NEW DISTOME *ENTEROHAEMATOTREMA* N.G. AND A NEW BLOOD FLUKE *HEMIORCHIS BENGALENSIS* N.SP. BELONGING TO THE FAMILY SPIRORCHIDÆ STUNKARD, AND A NEW SPECIES OF THE GENUS *DENDRITOBILHARZIA* SKRJABIN AND ZAKHAROW BELONGING TO THE FAMILY SCHISTOSOMATIDÆ POCHE, WITH REMARKS ON THE EVOLUTION OF THE BLOOD FLUKES

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(Received on April 6, 1940)

SUMMARY

A new genus *Enterohæmatotrema* belonging to the family Spirorchidæ from the intestine of a fresh water turtle is described and its affinities discussed.

The diagnosis of the genus *Hemiorchis* Mehra with an account of its two species is given. A new species of the little known genus *Dendritobilharzia* is described and two new subfamilies Dendritobilharzinæ and Gigantobilharzinæ are created.

A discussion on the evolution of the blood flukes is given.

The new genus *Enterohæmatotrema* described in this paper shows unmistakably close affinities with the blood flukes of the genus *Coeuritrema* Mehra, and represents the probable intestinal distome ancestor from which the blood flukes have been evolved. It falls into the group of the two genera of the subfamily Hapalotreminæ, *Cœuritrema* Mehra and *Hapalorhynchus* Stunkard which are characterised by the presence of the two testes with the ovary between them. Byrd (1939) in his revision of the family Spirorchidæ follows Price in considering these genera as synonymous, but as already discussed by us (1939) we do not agree with this view, and the account of the three new species of *Hapalorhynchus* described by Byrd (1939) confirms our opinion. *Enterohæmatotrema* n.g., is distinguished from *Coeuritrema* by the median pre-equatorial position of the genital opening on ventral side of the body immediately behind the acetabulum, shape and position of the cirrus sac and metraterm and the habitat in the gut instead of the vascular system of the turtle.

The view advocated by Byrd (1939) that the family Spirorchidæ should be considered as a unit and be not divided into subfamilies does not commend to us. Our system of classification is essentially based on the central idea of relationships displayed by the various subdivisions into which a group may be conveniently

divided with or without intergradations. If we can delimit the various subfamilies of the Spirorchidæ on the basis of fairly deep-seated and constant characters, irrespective of the intermediate characters displayed by some of the genera, there is no reason why this classification which is essentially meant for the convenience of workers on the group should not be adopted.

Byrd mainly on the basis of the reasons given by Stunkard in 1923 expresses his agreement with the latter author's view that the Spirorchidæ are the descendants of the more primitive Aporocotylidæ through the genus *Aporocotyle*. This view was fully discussed and refuted by us in 1933, and the converse view was put forward that the Aprocotylidæ and the more degenerate Sanguinicolidæ are the suckerless descendants of the Spirorchidæ. The phylogenetic scheme given by Byrd showing the probable relationship among the various genera of the Spirorchidæ is retrograde and untenable in view of our above-mentioned discussion, and this is confirmed now by the discovery of *Enterohæmatotrema* n.g., which while possessing Cœuritrema-Hapalorhynchus type of morphology is an intestinal and not a blood parasite of its host.

It is strange that Byrd considers *Plasmiorchis* to be synonymous with *Spirorchis*. The presence of a ventral sucker and the forwardly directed loops at the origin of the cæca are such constant features of the former genus so as to sharply separate it from the latter. *Hemiorchis bengalensis* n.sp. described in the paper confirms the validity of the genus *Hemiorchis* Mehra, 1939 created for *Plasmiorchis hardellii*.

A new species of the little known genus *Dendritobilharzia* from *Nettion crecca crecca* is described. The genera *Dendritobilharzia* and *Gigantobilharzia* are removed from the subfamily Bilharziellinæ to the two new subfamilies created for them, *i.e.*, the Dendritobilharzinæ and Gigantobilharzinæ respectively.

The genus *Chinhuta* Lal is dropped and its species is assigned to *Bilharziella* under the name *Bilharziella indica* (Lal, 1937).

## Family Spirorchidæ Stunkard.

### Subfamily Hapalotreminæ Stunkard.

### *Enterohæmatotrema* nov. gen.

*Generic diagnosis.* Spirorchidæ. Hapalotreminæ Minute, slender, delicate, elongated distomes; cuticle entirely devoid of spines or verrucæ. Oral sucker oval, somewhat cup-shaped, and protrusible; ventral sucker present at about one third body length from anterior end. Pharynx absent; œsophagus long and sinuous with two or three bends and surrounded by gland cells; œsophageal vesicle at intestinal bifurcation absent; intestinal cæca lateral, covered by vetellarian follicles behind genital opening and undulating behind posterior testis, terminating a short distance

in front of hinder end. Genital opening median, ventral, immediately behind ventral sucker and much in front of the middle of body length. Testes two in number with ovary between them, entire, subspherical or ovoid, situated in posterior half of body, immediately behind cirrus sac. Ovary transversely elongated or somewhat oval, situated between testes mostly to left side. Receptaculum seminis pear-shaped, to right side immediately in front of posterior testis. Transverse vitelline ducts closely behind ovary and proximal end of uterus or metraterm ; vitelline reservoir to left side immediately behind left transverse vitelline duct. Cirrus sac large, elongated, nearly straight or slightly curved in semilunar manner, thin-walled, slightly muscular and situated with long axis parallel to length of body to right side between genital opening and anterior testis, containing internal seminal vesicle, pars prostatica with numerous prostate gland cells and short unarmed eversible cirrus. External seminal vesicle situated to right side outside basal part of cirrus sac. Metraterm long, muscular, narrower proximally and much broader, somewhat swollen near distal end, situated to left side opposite to cirrus sac ; uterus indistinguishable from proximal part of metraterm, containing only one long, slightly coiled tubular ovum of 0·325 mm. length and 0·02 mm. maximum breadth. Vitellaria well developed, overlapping intestinal cæca, extending from just behind ventral sucker to a short distance in front of hinder end, uniting mesially behind posterior testis to form a large post-testicular mass. Parasitic in small intestine of fresh water turtles.

*Type species :* *Enterohæmatotrema palæorticum* n. sp.

*Enterohæmatotrema palæorticum* n sp.

(Fig. 1)

Eight specimens of which four were mature were obtained from the small intestine of three turtles of the species *Lissemys punctata* Malcolm Smith, three from two hosts each and two from the third host. This parasite appears to be rare ; only three out of many hosts examined were found infected with it. They were more or less intimately attached to the intestinal wall and took about half an hour to come out when the intestine was opened in salt solution, in which they moved actively by lashing movements like small nematode worms. Body minute, slender, elongated and delicate, usually tapering towards both ends, posterior end pointed ; short anterior part of body in front of ventral sucker narrow and prolonged as a neck region. Length of mature specimens 1·8—2 mm., of relatively immature specimens 1—1·2 mm. and maximum breadth 0·234 mm. in the region of testes and ovary. Body wall entirely devoid of spines or verrucæ. Oral sucker subterminal, oval or somewhat cup-shaped and protrusible like that of blood flukes, 0·06—0·072 mm in length and 0·042—0·051 mm. in breadth. Ventral sucker transversely elongated, 0·091—0·096 × 0·06—0·072 mm. in size, situated at about one-third to one-fourth body length from anterior end, *i.e.*, 0·44—0·57 mm. from it. Pharynx absent; œsophagus

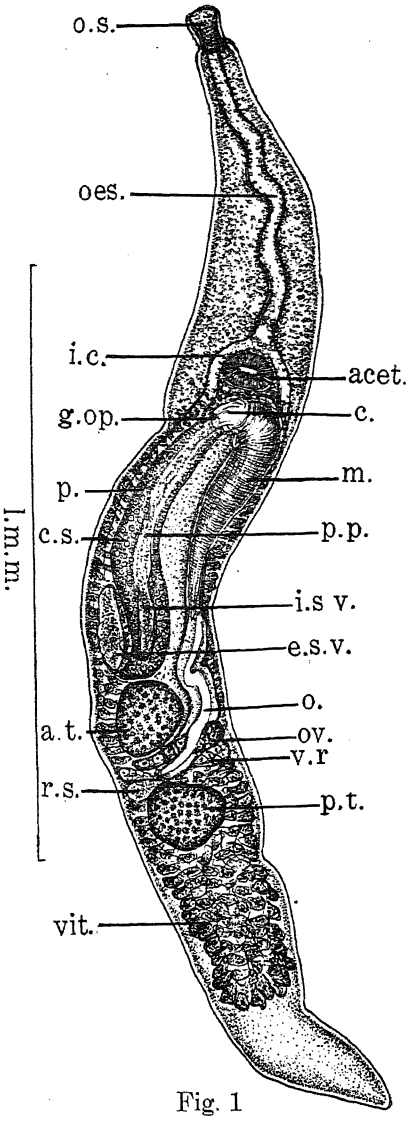


Fig. 1

Ventral view of *Enterohaematotrema palaeorticum* n. g. n. sp.

*acet.*, acetabulum; *a.l.i.c.*, anterior loop of intestinal caecum; *a.t.*, anterior testis; *c.*, cirrus; *c.s.*, cirrus sac; *d.c.*, diverticula of common caecum; *ex.b.*, excretory bladder; *e.s.v.*, external seminal vesicle; *e.v.f.*, extracaecal vitelline follicles; *g.op.*, genital opening; *g.l i.c.*, genital loop of right intestinal caecum; *g.l.l.i.c.*, genital loop of left intestinal caecum; *g.v.*, glandular vesicle; *i.c.*, intestinal caecum; *i.s.v.*, internal seminal vesicle; *m*, metraterm; *mo.*, mouth; *o.*, ovum; *oes.*, oesophagus; *oes.v.*, oesophageal vesicle; *o.v.*, ovary; *o.s*, oral sucker; *p.*, prostate gland cells; *p.p.*, pars prostatica; *p.t.*, posterior testis; *r.s.*, receptaculum seminis; *s.g.*, salivary gland cells; *s.m.*, shell gland mass; *t.*, testis; *ut.*, uterus; *u.v.d.*, unpaired vitelline duct; *vit.*, vitellaria; *vit.f.*, vitelline follicles; *v.r.*, vitelline reservoir.

long and sinuous with two or three bends, slightly dilated at its end just in front of intestinal bifurcation and surrounded by a large number of gland cells, measuring 0·4—0·52 mm. in length and 0·021– 0·03 mm. in greatest breadth ; median œsophageal vesicle behind origin of intestinal cæca at intestinal bifurcation characteristic of *Spirorchis* and *Plasmiorchis* absent ; intestinal bifurcation just in front of ventral sucker ; intestinal cæca pass laterally one on each side of ventral sucker, narrow confined to lateral edges of body up to the hinder end of posterior testis and covered by vitellaria, terminating at 0·312—0·338 mm. distance, *i.e.*, one-sixth to one-eighth, body length in front of hinder end, undulating slightly behind testes but not converging mesially towards each other behind ventral sucker as in the genus *Cœuritrema*. Genital opening median on ventral side immediately behind ventral sucker, in front of middle of body length, at 0·52—0·62 mm. distance from anterior end in a specimen of 1·8—2 mm. length. Testes two in number with ovary between them, subspherical or somewhat ovoid, situated in posterior half of body immediately behind cirrus sac, slightly to right side in same line with one another ; anterior testis 0·52—0·58 mm. behind acetabulum, measuring 0·117—0·12 mm. in diameter or 0·117—0·12 mm. in size ; posterior testis slightly larger than anterior testis, situated 0·065 mm. behind the latter and 0·494—0·5 mm. in front of hinder end, measuring 0·135 mm. in diameter or 0·169 × 0·13 mm. in size. Ovary with a compact mass of ova of large size at its inner end, transversely elongated or somewhat oval, situated between testes to left side nearer anterior than posterior testis, measuring 0·06 × 0·036 mm. in size Receptaculum seminis thin-walled, pear-shaped and filled with sperms, situated to right side closely inside right intestinal cæcum, immediately in front of posterior testis and just behind transverse vitelline duct of that side, measuring 0·063 mm. in length and 0·036 mm. in maximum breadth. Laurer's canal not seen. Cirrus sac large, thin-walled slightly muscular, elongated, longer than that of *Cœuritrema*, nearly straight except the slightly curved basal part or slightly curved throughout its length in a crescent-shaped manner with concavity on its inner border, situated to right side with long axis parallel to body length between genital opening and anterior testis, measuring 0·52—0·6 mm. in length and 0·091—0·13 mm. in maximum breadth, at about middle of its length or a little above the base. External seminal vesicle thin-walled, somewhat U-shaped consisting of large oval or elliptical and narrow tubular parts slightly overlapping one another, situated to right side overlapping right intestinal cæcum near body wall a little in front of anterior testis outside basal part of cirrus sac, and filled with sperms, measuring 0·108—0·11 mm. in length and 0·036 mm. in greatest breadth. Internal seminal vesicle contained in basal part of cirrus sac, straight, narrow, filled with sperms, 0·12—0·195 mm. in length and 0·024—0·027 mm. in greatest breadth ; pars prostatica long, narrow, tubular, 0·338—0·36 mm. in length and 0·027 – 0·03 mm. in maximum breadth ; cirrus protrusible, short and somewhat conical when protruded, found inserted in the type specimen in distal end of metraterm,

measuring 0·05—0·065 mm. in length and 0·03 mm. in greatest breadth at the base; prostate gland cells numerous around seminal vesicle and proximal half of pars prostatica, small number around distal half of the latter. Ootype passes just behind ovary into uterus, which continues insensibly into large muscular metraterm, both forming together a prominent tube of great length, narrow proximally, broad and dilated at distal end, situated to left side parallel to anterior testis and cirrus sac, overlapping ovary and measuring 0·585 mm. in length, 0·052—0·078 mm. in greatest breadth at distal end and 0·026 mm. in narrowest diameter at proximal end. Shell gland cells absent. Only one ovum contained at a time in uterus or proximal part of metraterm; ovum long, narrow, bent twice or somewhat coiled, produced into a bluntly pointed process at each end, measuring 0·325 mm. in length and 0·021 mm. in greatest breadth. Vitellaria composed of moderately sized or large follicles overlapping and outside cæca, commencing immediately behind acetabulum and terminating a little distance, *i.e.*, 0·28 mm. in front of hinder end of body, confined to lateral edges of body near body wall in front of anterior testis, but united behind posterior testis to form a large post-testicular mass of large follicles filling the body in this region. A few follicles pass inwards behind acetabulum to unite vitellaria anteriorly in front of genital opening. Transverse vitelline ducts arise from vitellaria just behind ovary and closely in front of posterior testis; vitelline reservoir a pear-shaped mass of follicles filled with yolk granules, situated to left side immediately behind ovary.

Host.—*Lyssemys punctata* Malcolm Smith.

Location.—Small intestine.

Locality.—Allahabad, India.

Remarks:—The genus *Enterohæmatotrema* bears close resemblance to the blood flukes of the genus *Cœuritrema* Mehra on account of the absence of the pharynx, great length of the œsophagus, position of the testes with the ovary between them in the posterior half of body with the well developed cirrus sac, external seminal vesicle and metraterm in front, post-acetabular position of the genital opening in front of the gonads and far in front of the hinder end and similarity in the position of the receptaculum seminis and vitellaria. But it dffers remarkably in the median pre-equatorial position of the genital opening on ventral side of the body just behind acetabulum (in *Cœuritrema* genital opening is dorsal, sinistral, close behind acetabulum near middle of body length), in the shape and position of the cirrus sac and metraterm, which are much more elongated and lie parallel to one another along the long axis of the body, and above all in the habitat in the gut instead of the heart and arteries of the turtle. The new genus belongs to the subfamily Hapalotreminæ of the family Spirorchidæ on account of the general topography and falls into the group of the two genera *Cœuritrema* Mehra and *Hapalorhynchus* Stunkard on account of the presence of two testes separated by the ovary.

As discussed by us previously (1939), we do not agree with Byrd (1939), who follows Price (1934) in considering *Coeuritrema* to be synonymous with *Hapalorhynchus*, and the description of three new species of the latter genus by Byrd (1939) confirms our opinion. In *Hapalorhynchus stunkardi* Byrd the much larger vesicula seminalis occupies a different position from that in *Coeuritrema* and the large mass of large prostate gland cells surrounds the cirrus sac quite unlike that in the latter genus, in which the prostate gland cells lie within the cirrus sac. Moreover, the cirrus and metraterm are poorly developed and slightly muscular in *H. stunkardi*, reverse to that in *Coeuritrema*. The shape of the ovum also differs. The cirrus sac and metraterm are indistinguishable or poorly developed in *H. evaginatus* Byrd, but they are well developed in *Coeuritrema*. *H. reelfooti* Byrd, however, stands closer to the latter genus on account of the prostate gland cells being enclosed within the slightly muscular cirrus sac, but the cirrus seems to be poorly developed, the metraterm is absent, and the external seminal vesicle is much larger and occupies a different position.

Subfamily Spirorchinæ Stunkard.

*Hemiorchis hardellii* Mehra (1939) (syn. *Plasmiorchis hardellii* Mehra 1934).

(Fig. 2)

A number of these blood flukes were obtained recently from the ventricle of the heart and aortic arches of two *Hardella thurgi* at Allahabad. To the detailed account of this species given in 1934 a few more points are added.

Length 4—4·5 mm.; greatest breadth 1·05—1·12 mm. in acetabular region or a little behind it. Fine needle-like spines of 0·015 mm. length lie within cuticle of about the same thickness and do not project outside it. External seminal vesicle very small, rather inconspicuous, situated outside basal end of cirrus sac between hindmost testis and ovary. Cirrus sac large with thick muscular walls, situated obliquely to left side opposite ovary with basal end in level with or a little in front of it and terminal end surrounded by genital loop of left intestinal cæcum behind it, measuring 0·225 mm. in length and 0·108– 0·12 mm. in greatest breadth. Internal seminal vesicle long and much coiled; numerous prostate gland cells surrounding internal seminal vesicle and small rounded pars prostatica; ejaculatory duct or cirrus with muscular walls short and curved. Ovary small, lobed, situated to right side closely inside right cæcum, just in front of and sometime partly overlapping inwardly pointed bent end of genital loop of that cæcum, a little distance behind hindmost testis, broader than long, measuring 0·075—0·12 × 0·18 – 0·195 mm. in size. Ovum single, oval, without polar filaments, 0·075—0·08 × 0·027—0·03 mm. in size. Transverse vitelline ducts situated a little behind ovary; vitelline reservoir small, median, just in front of transverse vitelline ducts.

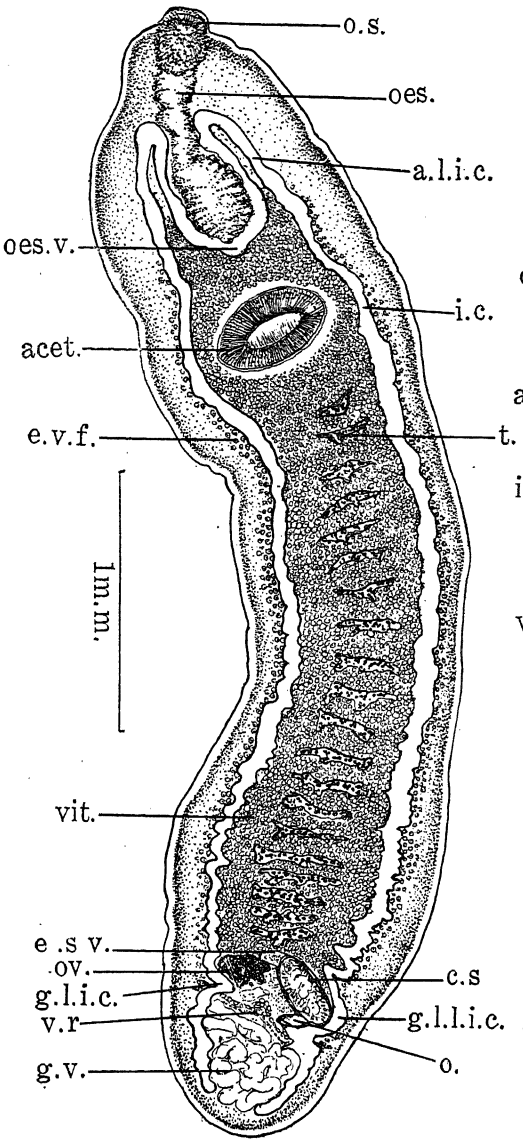


Fig. 2

Ventral view of *Hemiorchis hardellii* Mehra.

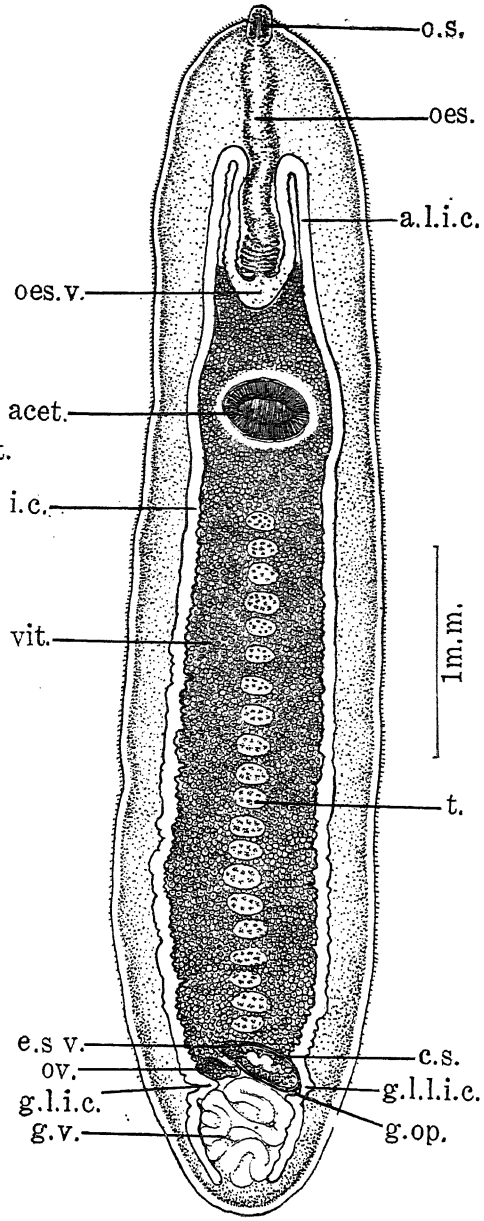


Fig. 3

Ventral view of *Hemiorchis bengalensis* n. sp.

(Lettering as in Fig. 1)

Vitellaria forming one large mass of numerous small rounded follicles occupying characteristically entire intercæcal region around acetabulum and testes from intestinal bifurcation or a little further in front to anterior margin of ovary and cirrus sac, and also composed of a small number of extracæcal follicles forming a narrow fringe immediately outside cæca. The entire intercæcal parenchymatous tissue around acetabulum and testes from the intestinal bifurcation and within basal parts of forwardly directed loops to the ovary is developed into a large mass of small deeply staining vittelline follicles, which forms a characteristic feature of the genus *Hemiorchis*. On a re-examination of the entire mounts on which our previous account of this species was based this description of the vitellaria is confirmed, and it must be admitted that the large characteristic intercæcal vitellarian mass described above was inadvertently overlooked at that time.

*Hemiorchis bengalensis* n.sp.

(Fig. 3)

Host.—*Hardella thurgi* Boulenger.

Location —Ventricle of heart.

Locality.—Rana Ghat, Bengal.

Four specimens of this blood fluke were collected by Mr. R. C. Chatterji from the ventricle of the heart of one fresh water tortoise, *Hardella thurgi* obtained from Rana Ghat, Bengal and dissected in the Zoological laboratory of the Allahabad University in April 1940. My best thanks are due to Mr. R. C. Chatterji for giving me two specimens for study.

Body thin, transparent, elongated, and elliptical with rounded anterior and posterior ends; anterior end broader. Length in entire mounts 6 mm.; maximum breadth 1·27 or 1·035 mm. at a little behind middle of testicular region; breadth in acetabular region 1·11 or 0·81 mm.; breadth in region of ovary 0·99 or 0·705 mm. respectively in the two specimens examined. The body is narrower and more elongated than in type species. Body wall armed with minute pointed denticles or spines projecting outside thin cuticle unlike those in the type species. Oral sucker oval, longer than broad, slightly protrusible, 0·204 or 0·188 mm. in length, 0·155 or 0·132 mm. in maximum breadth respectively. Acetabulum stouter, slightly broader than long, about one and a half time larger than oral sucker, 0·27×0·333 and 0·237×0·25 mm. in size in the two specimens respectively, and situated at 1·65—1·75 mm. from anterior end, *i.e.*, at about one-third body length from the latter. Pharynx absent; œsophagus long and slightly undulating with two bends, 1·215 and 1·125 mm. in length in the two specimens, *i.e.* about one-fifth part of the body length, and uniform in breadth except at ends; deeply staining salivary gland cells prominently developed and much larger in number around terminal part of œsophagus; œsophageal vesicle larger and more prominent than that in *Hemiorchis hardellii*.

Intestinal cæca arise laterally from œsophageal vesicle and pass anteriorly as forwardly directed loops lying parallel to œsophagus for two-third to three-fourth of its length; behind acetabulum cæca provided with indented margins or minute irregular diverticula specially on inner side; genital loops well developed as in type species, that of left cæcum semicircular and wider with genital opening inside it and that of right cæcum with bent end directed inward just behind ovary. Glandular vesicle in the form of a large convoluted tubular mass filling entire intercæcal space behind ovary and cirrus sac, in the region marked anteriorly by genital loops and pressed laterally against walls of cæca. Excretory opening dorsal a little in front of hinder end; excretory bladder V-shaped, bend of the V broad and situated just behind glandular vesicle and blind ends of cæca, narrow tubular cornua not seen beyond the latter on account of their being overlapped by cæca.

Testes 20 in number and arranged in a regular linear series in median line, separated from one another by a little distance and from cæcum on either side by a considerable distance, commencing 0·52 or 0·36 mm. behind acetabulum, oval in shape with entire margins and broader than long, 0·126—0·135×0·09 mm. in size in fully mature specimen, and smaller and somewhat rounded, 0·05—0·06× 0·045 mm. in less mature specimen. The new species differs from *H. hardellii* in the shape and size of testes. Vesicula seminalis externa moderately sized, somewhat oval, 0·07—0·12×0·015—0·075 mm. in size, filled with sperms, and situated slightly to right side between hindmost testis and ovary partly overlapping basal end of cirrus sac. Cirrus sac large with thick muscular walls, situated semiobliquely to left side just behind hindmost testis and immediately in front of glandular vesicle with basal end in median line just in front of ovary and terminal end a little behind the latter, measuring 0·288—0·3 mm. in length and 0·081—0·12 mm. in maximum breadth. Internal seminal vesicle long and slightly coiled surrounded by numerous prostate gland cells; pars prostatica small; muscular ductus ejaculatorius or cirrus short and curved. Genital opening on left side just inside left cæcum within its genital loop, at 0·55—0·6 mm. in front of hinder end.

Ovary subspherical with a small oval mass of large ova protruding from its inner side, transversely elongated, 0·159×0·09 mm. in size inclusive of the inner mass of large ova of 0·066×0·045 mm. size, situated to right side closely inside and touching inner margin of right cæcum just in front of bend of right genital loop, 0·09 mm behind hindmost testis and at about one-ninth part of body length from hinder end; oviduct arising from hinder end of inner margin of ovary runs backwards; receptaculum seminis rounded, situated a little behind ovary to right side, 0·06 mm. in diameter; shell gland cells and Laurer's canal not seen; metraterm present; ovum single, large, oval, without polar filaments, 0·09 mm. in length and 0·06 mm. in greatest breadth. Vitellaria exclusively intercæcal, forming one huge

mass of numerous small rounded follicles around acetabulum and testes from intestinal bifurcation or origin of œsophageal vesicle to ovary, external seminal vesicle and cirrus sac. The entire intercæcal parenchymatous tissue in front of the latter organs is developed around and in front of the testes into the large mass of vitelline follicles. In this species the vitelline follicles are entirely absent outside the cæca. Transverse vitelline ducts lie just in front of glandular vesicle behind ovary, with small vitelline reservoir in front more or less in median line.

Remarks.—*Hemiorchis bengalensis* n. sp. is distinguished from *Hemiorchis* (syn. *Plasmiorchis*) *hardellii* Mehra by the narrow and more elongated shape of the body, cutaneous spines projecting outside the cuticle, larger size of the œsophageal vesicle, shape of the testes (oval with entire margins in *bengalensis*, narrow, transversely elongated and irregularly lobed in *hardellii*), moderate size of the external seminal vesicle, shape of the ovary, exclusively intercæcal position of the vitellaria and difference in size of the various organs.

*Hemiorchis* Mehra, 1939.

*Generic diagnosis.*—Spirorchidae. Spirorchinae. Hermaphrodite distome blood flukes. Body small, thin, elongated, flattened, somewhat elliptical with rounded anterior and posterior ends; cuticular spines present. Oral sucker oval, longer than broad, slightly protrusible; acetabulum stouter and larger than oral sucker, broader than long, situated at about one-third body length from anterior end. Pharynx absent; oesophagus long, sinuous, surrounded by numerous salivary gland cells near its distal end; oesophageal vesicle present; intestinal cæca with forwardly directed loops one on each side of oesophagus, terminating near hinder end of body, and forming well marked genital loops, right loop short and bent inwards just behind ovary and left loop semicircular just outside genital opening. Glandular vesicle in the form of a large convoluted tubular mass filling entire intercæcal space near hinder end behind ovary and genital opening. Excretory opening dorsal, a little in front of hinder end; excretory bladder V-shaped, bend of the V broad and situated just behind glandular vesicle and blind ends of cæca. Genital opening ventral to left side, closely inside left cæcum in its genital loop, just behind ovary and a short distance in front of hinder end. Testes large in number, arranged in a regular linear series in median plane between cæca a short distance behind acetabulum and in front of ovary. External seminal vesicle small or moderate sized. Cirrus sac large with thick muscular walls, situated obliquely or semi-obliquely to left side behind hindmost testis opposite ovary, enclosing long, coiled internal seminal vesicle and small pars prostatica surrounded by numerous prostate gland cells and short ductus ejaculatorius or cirrus. Ovary small, to right side closely inside right cæcum just in front of small right genital loop, a little distance in front

of hinder end; receptaculum seminis and metraterm present. Vitellaria mostly forming one characteristic large intercæcal mass of numerous small rounded follicles around and in front of acetabulum and testes from intestinal bifurcation or a little in front to ovary and a narrow fringe of extracæcal follicles just outside cæca, or exclusively intercæcal forming the large characteristic mass mentioned without fringe of extracæcal follicles; transverse vitalline ducts just behind ovary with small median vitelline reservoir just in front. Ovum single, large, oval, without polar filaments. Parasitic in ventricle of heart and arteries of fresh water turtles, India.

Type species: *Hemiorchis hardellii* Mehra, 1939 (syn. *Plasmiorchis hardellii* Mehra, 1934).

Family Schistosomatidæ Poche.

Subfamily Dendritobilharzinæ n. subf.

*Dendritobilharzia asiaticus* n. sp.

(Figs. 4 & 5)

One female blood fluke of this species was obtained from a branch of the anterior mesenteric vein of the common teal, *Nettion crecca crecca* caught at Allahabad. The male is unknown. Body small, elongated, flattened, somewhat tongue-shaped, narrower at ends with more or less rounded anterior and bluntly pointed posterior ends. Length 6 mm., maximum breadth 1·3 mm. immediately behind posterior union of cæca, breadth in region of ovary 1·26 mm.; cuticle thin, devoid of spines or tubercles. Suckers absent. Mouth subterminal, ventral, near anterior end, rounded, very small, 0·012 mm. in diameter, armed internally with very minute cuticular denticles; pharynx absent; œsophagus 0·5 mm. long, surrounded by salivary gland cells forming a relatively thin layer around anterior third and a thick bulb-shaped mass around posterior third of its length; œsophageal bifurcation at 0·52 mm. from anterior end. Intestinal cæca narrow near their origin and their posterior union, sinuous, provided with a few short lobe-like or tubular outgrowths; posterior union of cæca at 1·2 mm. from œsophageal bifurcation and at about one-fourth body length from anterior end; common cæcum long, zigzag, branched, reticular and provided with short lateral club-shaped, tubular diverticula, which intermingled with and surrounded by numerous vitelline follicles from a large complicated mass filling almost the entire body behind posterior union of cæca.

Genital opening terminal at anterior end, close and to right side of oral opening. Ovary intercæcal, situated mostly to right side near right cæcum a little in front of posterior union of cæca, at 1·05 mm. distance behind anterior end, very long and much coiled, consisting of seven large loosely arranged spiral coils placed closely behind one another, measuring 2·8 mm. in length and 0·063 mm. in greatest breadth; anterior or proximal four coils composed of primordial ova pressed against one

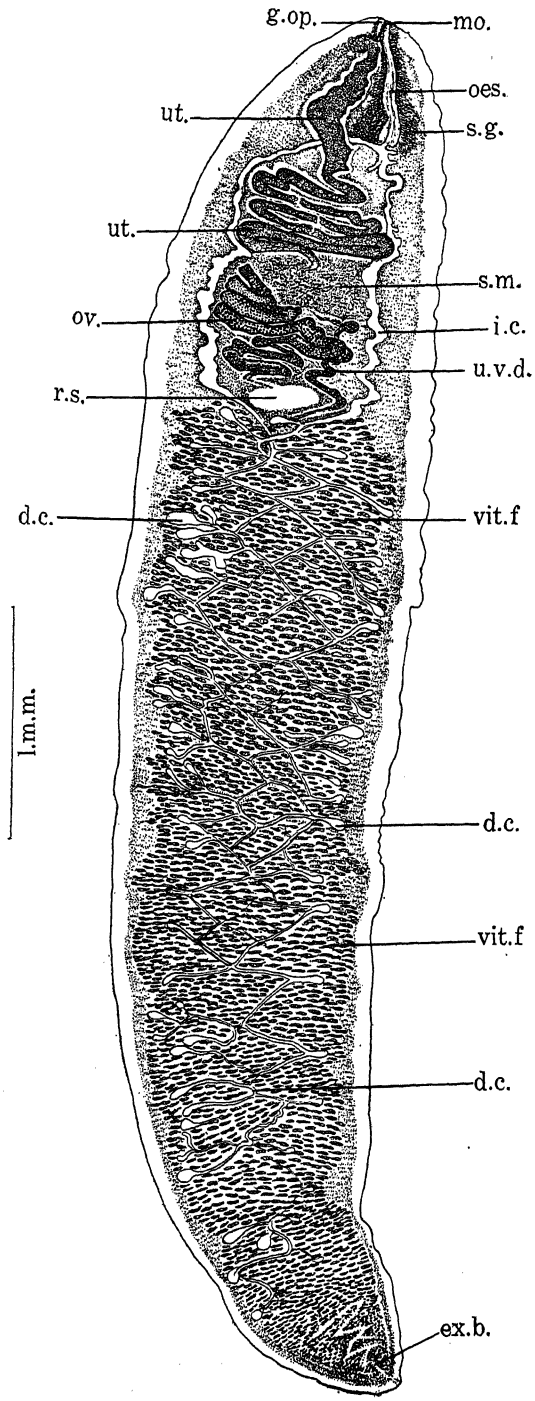


Fig. 5

Fig. 4.—Ventral view of female *Dendritobilharzia asiaticus* n. sp.
Fig. 5.—Ovum of *Dendritobilharzia asiaticus* n. sp.
(Lettering as in Fig. 1)

another, posterior or distal three ovarian coils short, narrow, and composed of 4—6 rows of large mature ova. Oviduct a continuation of narrow posterior end of ovary; receptaculum seminis oval, 0·24 × 0·09 mm. in size, situated median behind ovary just in front of posterior union of cæca, with oviduct at its anterior margin slightly to right side. Vitellaria unpaired, composed of numerous pear-shaped follicles mixed up with and surrounding dendritic diverticula of the common cæcum, occupying almost entire body from cæcal union to posterior end; unpaired vitelline duct prominent, sinuous, in intercæcal space contiguous and to right side of posterior four coils of ovary, arising from vitelline mass at posterior union of cæca. Uterus large, convoluted, arising from anterior margin of shell gland mass near median line at about 0·52 mm. behind œsophageal bifurcation, composed of four large convolutions filling entire intercæcal space a little behind œsophageal bifurcation and passing forwards as a large compact mass of ova in front of the latter to right side of œsophagus to open at the genital opening. Ova numerous, with fairly thick yellow brown shell, nearly oval and produced at one end into a short pointed spine measuring 0·027—0·033 mm. in length including the spine, and 0·015—0·018 mm. in maximum breadth.

Host.—*Nettion crecca crecca* Stuart Baker.

Location.—Branch of anterior mesenteric vein.

Locality.—Allahabad.

Remarks:—The genus *Dendritobilharzia* was created in 1920 by Skrjabin and Zakharow for *Dendritobilharzia odhneri*, which was later (1924) recognised by Skrjabin to be identical with *Bilharziella pulverulenta* Braun, 1901. Both the accounts of the type species *Dendritobilharzia pulverulenta* (Braun, 1901) Skrjabin, 1924 by Braun and Skrjabin and Zakharow were based on the description of the male specimen obtained from *Querquedula querquedula* (*Anas querquedula*) in Africa (Dongola, Sudan) and Europe (Russia). The female of this species, which is much smaller than the male, *i.e.*, 1·5657 mm. long by 0·2875 mm. wide, was described by Semenov from *Anas platyrhynchos* (*A. boschas*) in 1927 from Russia. The second species of this genus, *Dendritobilharzia loossi* Skrjabin was described by Skrjabin in 1924 from the female specimen obtained from *Pelecanus onocrotalus* in Russia (Europe). The female of this species which is much larger in size than that of the type species is also inadequately described. Though Skrjabin's paper (1924), giving its description is inaccessible to me, the meagre account reproduced by Price (1929) mentions the absence of oral sucker and acetabulum, 0·45 mm. as the length of the œsophagus, position of the posterior union of the cæca about 3·47 mm. from the œsophageal bifurcation, common cæcum as in *D. pulverulenta*, the spiral tubular ovary and unpaired vitelline duct in the space between intestinal cæca and vitelline follicles situated along the course of the common cæcum. The new species which is described from a female specimen and represents the third species of the genus,

is easily separated from the type species by the much larger size of the female, the body not divided into two parts by an irregular transverse groove, and the large size and coiled form of the ovary (ovary 2·8 mm. long by 0·063 mm. broad in the new species, 0·1028 mm. long by 0·0914 mm. in *D. pulverulenta*). It differs from *D. loossi* in size (female 14·2 mm. long by 1·41 mm. wide in *loossi* and 6 mm. long by 1·3 mm. broad in *asiaticus* n. sp.), in the posterior union of the cæca at 1·2 mm. from the œsophageal bifurcation (in *loossi* 3·47 mm. from œsophageal bifurcation), in the coiled form of the ovary and the uterus filled with numerous ova. It may also be remarked that the large convoluted uterus filled with numerous ova and terminal position of the genital opening at the anterior end in the female, which are so characteristic of the new species have not been observed in *D. loossi*.

We exclude the genus *Dendritobilharzia* from the subfamily Bilharziellinæ and create a new subfamily Dendritobilharzinæ for it on account of the long common cæcum provided with lateral dendritic branches, large convoluted uterus filled with numerous ova, anterior terminal position of the genital opening in the female and numerous vitelline follicles along with lateral branches of the common cæcum almost filling the whole body behind the cæcal union. It appears that from the form like *Bilharziella* have been evolved along one line of evolution the genera of the subfamily Schistosomatinæ and along the other two separate lines the genera *Dendritobilharzia* and *Gigantobilharzia*. The genus *Chinhuta* Lal, 1937 resembles *Bilharziella* closely except in the presence of a gynæcophoric canal, which in our opinion is absent in it. According to Lal the lateral edges of body in *Chinhuta* are rolled inwards to form a deep gynæcophoric groove, which extends right from the hinder end of the oral sucker to the posterior end. The gynæcophoric canal in the subfamily Schistosomatinæ, as is well-known, extends from behind the acetabulum and not from behind the oral sucker and, moreover, Lal does not mention whether he found any female specimen enclosed in the gynæcophoric canal of the male, specially when he collected about 200 specimens from the same host. Arnaldo Giovannola (1936) even in unisexual infection with *Schistosoma mansoni* found three couples, all males, in the copula position, and the three males observed in the gynæcophoric canals of other males were of smaller size. So Lal's observation on the presence of a gynæcophoric canal in *Chinhuta* is not at all convincing, on the other hand what he calls by this name appears to be a depression on the ventral side, which ordinarily occurs in some distomes due to contraction during fixation or the dying state of the worm. The position of the ovary in the figure by Lal outside the common cæcum just behind the posterior cæcal union requires to be confirmed, as its usual position in the family is intercæcal in front of the posterior union of the cæca. The morphology of both the male and female specimens of *Chinhuta* resembles so closely that of *Bilharziella*, that we are constrained to drop this genus and assign its species to the latter genus as *Bilharziella indica* (Lal, 1937) Mehra, 1940.

Dendritobilharzinæ n. subf.

*Subfamily diagnosis.*—Schistosomatidæ. Body of both sexes elongated, flattened and similar in form. Gynæcophoric canal absent. Suckers absent. Paired intestinal cæca short; common cæcum long and provided with lateral dendritic branches. Genital opening in male in anterior part to left side just in front of posterior cæcal union, in female terminal at anterior end. Testes numerous, about 110 in number, situated in a zigzag line along the common cæcum from cæcal union to posterior end. Ovary spiral or coiled, intercæcal; uterus large, coiled and filled with numerous ova. Vitelline follicles numerous, mixed up with dendritic branches of common cæcum, filling almost entire body behind posterior cæcal union.

Type genus.—*Dendritobilharzia* Skrjabin and Zakharow, 1920.

Gigantobilharzinæ n. subf.

*Subfamily diagnosis.*—Schistosomatidæ. Body of both sexes very long, elongated, cylindrical, female shorter than somewhat flattened male; posterior extremity of both sexes provided with lateral lobe-like projections. Oral sucker usually absent, seldom present. Ventral sucker always absent. Gynæcophoric canal absent or rudimentary. Paired intestinal cæca short; common cæcum very long without lateral branches. Genital opening in male between œsophageal bifurcation and cæcal union, in female median much in front of œsophageal bifurcation, near anterior end. Testes very numerous, more than four hundred in number, from behind cæcal union to hinder end. Ovary moderately long, spiral. Vitelline follicles occupying about nine-tenths of body length. Uterus short containing a few ova.

Type genus.—*Gigantobilharzia* Odhner, 1910.

The subfamily Bilharziellinae Price, 1929 is retained for the genera *Bilharziella* Looss, 1899 and *Trichobilharzia* Skrjabin and Zakharow, 1920.

## Remarks on the Evolution of the Blood Flukes

Odhner (1912) while discussing the affinities of *Hapalotrema* with *Liolope*, *Bilharziella*, *Ornithobilharzia*, and *Bilharzia* traced their origin from the intestinal distome, *Liolope* Cohn. He derived the *Bilharzia* type from the blood fluke *Hapalotrema*, which he included with the intestinal distomes *Liolope* and *Helicometra* in the subfamily Liolopinae, family Harmostomidae, and deferred to a subsequent paper a discussion of the affinities of the blood flukes of fishes, the Aporocotyle-Sanguinicola series. Stunkard (1921 and 23) on the basis of a close similarity of *Spirorchis* with *Aporocotyle* recognised that the Aporocotylidae of fishes, the Spirorchidae of turtles and the Schistostomidae of birds and mammals from a well defined group with inherent natural relationships. He put forward the view that the Spirorchidae stand in an intermediate position, and that the Schistosomes are to

be derived through them from the Aporocotylidae rather than from the Harmostomidae as maintained by Odhner. According to him the blood flukes originating with the most degenerate suckerless forms with reduced alimentary tract, *Sanguinicola* and *Aporocotyle* were evolved through *Spirorchis* and *Hapalotrema* to *Schistosomes*. Poche (1925) did not accept Stunkard's view saying that the absence of suckers and the presence of follicular testes in the Aporocotylidae and Sanguinicolidae warrant against it.

La Rue (1926) established that the Strigeidæ, Schistosomatoidea and Gasterostomata are genetically related, and accordingly proposed a single order Strigeatoidea to include them under the three suborders Strigeata, Schistosomata and Bucephalata. Szidat (1928) mainly on the basis of the life history studies pointed out the affinities of the Harmostomidæ (*Harmostomum leptostomum*, *Urogonimus macrostomum*), *Clinostomum* and *Sphærostomum* with the La Rue's order Strigeatoidea, mentioning that they have secondarily lost the tailed cercarial larval stage in their development. He finds himself in agreement with Odhner on the phylogeny of these forms based on the comparative anatomy studies. According to him the ancestral form of the Strigeatoidea, Sanguinicolida, Harmostomidea, Clinostomidea stands nearest to *Sphærostoma bramæ*, from which he derives La Rue's suborders along two lines of evolution, one passing through *Liolope copulans* to the blood flukes of reptiles, birds and mammals and the other passing into the suborder Strigeata, the immediate hypothetical ancestor of which is not known to be connected with any living representative. He thinks that the Bucephalata originated at the root of the Strigeata branch and are closely related to its primitive genera *Cyathocotyle* and *Prohemistomum*, with which they show much resemblance in the position of the genital organs and the presence of a cirrus sac.

We in 1933 supported Odhner's view about the orgin of the Schistosomatidæ from *Hapalotrema* through *Liolope* and postulated that the subfamily Hapalotreminæ of the Spirorchidæ formed the central stock from which have been evolved along one line the Spirorchinæ, and through *Spirorchis* the Aporocotylidæ and the more degenerate Sanguinicolidæ, and along the other line the Schistosomatidæ. *Coeuritrema* was considered to represent the blood fluke closely related to the ancestor through which the Hapalotreminæ have been evolved from the Liolopinæ. We rejected Stunkard's and Ejsmont's view about the phylogeny of blood flukes in an ascending series from the Aporocotylidæ, through the Spirorchinæ to the Hapalotreminæ and Schistosomatidæ on the analogy of various groups of animals that evolution always takes place in divergent lines from a central generalised type. Byrd (1939) without giving any arguments supports Stunkard that the Spirorchidæ are descendants of the more primitive Aporocotylidæ through the genus *Aporocotyle* to the genus *Spirorchis*. He gives a schematic diagram showing the probable phylogenetic

relationship among the various genera of the Spirorchidæ and with other blood flukes, which is retrograde and cannot be accepted.

There is no doubt that the blood flukes have been evolved from the intestinal distomes in the early phylogenetic history of the Digenea. The discovery of *Enterohæmatotrema* n.g. which is parasitic in the gut of turtles and is closely related to the blood flukes of the genus *Cœuritrema* adds suport to this view. The blood flukes have been parasitic in vertebrates for a long time. We are of opinion that they originated in the reptilian hosts as the Hapalotremine ancestors of the Spirorchidæ being closely related to *Enterohæmatotrema* n.g., *Cœuritrema* and *Hapalorhynchus*, a view already discussed by us in 1933. Stunkard, Ejsmont and Byrd, however, trace their origin from the suckerless degenerate forms belonging to the Aporocotylidæ parasitic in piscine hosts, mainly because the fishes are the oldest and most primitive vertebrates, and the most highly specialised blood flukes, the dioecious forms are parasitic in the most recent and highly specialised vertebrates, the birds and mammals. It has been already discussed that the morphological features involved in tracing the phylogeny of blood flukes are the number of testes, two or many, their relation to the ovary, the position of the genital opening behind the testes near the hinder end or in front of them a little behind the acetabulum, in front of or at about the middle of body length, and the presence of a well developed cirrus sac and metraterm. There is no doubt that the ancestor of blood flukes, which is represented in the intestinal distome *Enterohæmatotrema* possessed two testes, which is the most usual number for the Digenea. The division of two testes into a large number is a secondary condition met within certain genera of a few other families besides the families of blood flukes. We maintain that in the totality of organisation the generalised Fasciolid ancestor of blood flukes resembled closely *Enterohæmatotrema* and not *Aporocotyle* or *Spirorchis* as Stunkard holds. The primitive position of the genital opening in the order Fasciolata should be considered to be near the acetabulum, *i.e.*, a little in front of or behind it, but not much behind it near the hinder end. In the position of the ovary between the two testes, the ancestor of blood flukes symbolised in *Enterohæmatotrema* resembled the families of the Fasciolata, Harmostomidæ syn. Brachylæmidæ and Clinostomidæ. We, therefore, agree with Szidat that the blood flukes are evolved from the common Fasciolid ancestor of the order Stigeatoidea La Rue along one line of evolution, originating in the Liolopinæ (Harmostomidæ) which were parasitic in the reptiles developing out of amphibians. *Enterohæmatotrema-Cœuritrema-Amphiorchis* series represents the central group of the Spirorchidæ which were evolved along one line, as discussed by us before, into the Schistosomatidæ, along another line through *Hapalotrema* and *Learedius* into *Aporocotyle* *i.e.*, the Aporocotylidæ and Sanguinicolidæ while the third branch originating in *Learedius* became evolved into the remaining genera of the Spirorchinæ, *i.e.*,

*Monticellius*, *Hemiorchis*, *Plasmiorchis* and *Spirorchis*. The view advocated by Stunkard and Ejsmont and accepted by Byrd without comment that the blood flukes have been evolved in an ascending series from the Aporocotylidæ through the Spirorchinæ to the Hapalotreminæ and the Schistosomatidæ in co-relation with the evolution of their hosts from the most primitive vertebrates, the fishes to the most highly specialised vertebrates, the birds and mammals through the reptiles is against the fundamental concept of comparative morphology of the blood flukes and other digenetic trematodes and the evolutionary hypothesis. Evolution does not take short cuts along one line as this view contemplates. It is our conviction that the blood flukes originated in the amphibian and reptilian hosts as members of the Spirorchidæ and from the reptiles they passed on into the avian and mammalian hosts along one line as members of the Schistosomatidæ and into the piscine hosts along the other line of evolution as members of the Aporocotylidæ and Sanguinicolidæ.

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# FURTHER STUDIES ON THE EFFECT OF ALCOHOL ON THE RESPIRATORY RATE OF LEAVES

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## SUMMARY

In this paper the effect of alcohol on the respiratory rates of the leaves of *Mangifera indica* and *Allium tuberosum* has been described. The experimental results indicate that the respiratory rate increases with the increase in the concentration of alcohol introduced into the leaves; beyond a certain concentration, however, alcohol brings about a fall in the carbondioxide output. It has been found that the acceleration of carbondioxide production by alcohol decreases with time. Lower percentages of alcohol maintain an increased respiratory rate for a longer interval of time than comparatively stronger solutions. The results are in general agreement with those obtained with the leaves of *Eugenia jambolana* described in another paper.

## Introduction

The effect of alcohol on the respiratory rate of *Eugenia jambolana* leaves has been described in a previous paper[1]. In order to find out whether foliage organs of other plants respond in a similar manner to alcohol injections, experiments were performed with the leaves of *Mangifera indica* and *Allium tuberosum*. The results obtained have been analysed in a similar way as in the case of *Eugenia jambolana*.

## Materials and Methods

Mature green leaves of *Mangifera indica* were chosen for this work; care was taken to ensure that the leaves selected for different experiments were of about the same age. For selecting *Allium tuberosum* leaves, the foliage organs were cut at about two inches from the ground. The older leaves could easily be distinguished by their yellow and shrivelled tips and could therefore be easily discarded. The younger healthy leaves were chosen as materials for this work.

The respiratory current was drawn through Pettenkoffer tubes containing standardised barium hydroxide solution which was ultimately titrated. Injection was carried out by means of a vacuum pump and the alcohol used was ethyl

TABLE I

*Mangifera indica*

Mgms. of $CO_2$ per 10 gms. of leaves

| Hours | Before injection | | | | | | | After injection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3-6 | 6-9 | 9-12 | 12-15 | 15-18 | 18-21 | 21-24 | 24-27 | 27-30 | 30-33 | 33-36 | 36-39 | 39-42 | 42-45 | 45-48 | 48-51 |
| Treatment ... | | | | | | | | | | | | | | | | |
| Air ... ... | 29·3 | 31·2 | 30·0 | 27·5 | 22·8 | 21·2 | 20·3 | 20·4 | 20·4 | 20·0 | 19·2 | 18·8 | 18·4 | 17·6 | 17·0 | 17·4 |
| Water ... | 28·0 | 32·4 | 30·8 | 25·6 | 23·0 | 21·2 | 20·8 | | 23·0 | 20·2 | 19·0 | 18·9 | 18·4 | 18·0 | 17·0 | 17·3 |
| 2% alcohol ... | 29·0 | 31·8 | 30·0 | 25·0 | 22·6 | 21·2 | 20·8 | | 23·5 | 22·5 | 20·6 | 19·6 | 19·0 | 17·6 | 17·9 | 17·9 |
| 5% „ ... | 29·0 | 32·5 | 30·8 | 28·0 | 24·0 | 22·8 | 21·8 | Injected here | 26·2 | 25·2 | 22·6 | 21·0 | 21·4 | 19·2 | 18·8 | 18·6 |
| 8% „ ... | 31·0 | 33·2 | 31·6 | 28·0 | 23·2 | 21·4 | 21·4 | | 27·2 | 28·2 | 25·2 | 21·0 | 21·8 | 19·4 | 19·4 | 18·4 |
| 10% „ ... | 32·0 | 32·6 | 29·3 | 27·8 | 22·4 | 20·3 | 20·7 | | 18·0 | 31·2 | 24·5 | 20·7 | 18·0 | 16·7 | 16·2 | 16·0 |
| 15% „ ... | 26·3 | 31·0 | 30·2 | 27·3 | 22·6 | 22·0 | 21·4 | | 17·2 | 15·1 | 12·6 | 14·0 | 14·5 | 15·0 | 11·3 | 8·6 |

alcohol ($CH_3CH_2OH$, Merck). The control sets of leaves were injected with distilled water. The various alcohol solutions also were made with distilled water. The leaves of *Mangifera indica* were allowed to respire normally for 24 hours and those of *Allium tuberosum* for 9 hours before being subjected to alcohol and water injections. For fuller details regarding experimental procedure, reference should be made to the paper on *Eugenia jambolana*[1].

## Experimental Results and Discussion

The results of different experiments are given in Table I and II; in the case of water-injected leaves, instead of separate carbondioxide values in each experiment, an average respiratory rate has been given in the tables for comparison.

### Table II

*Allium tuberosum*

Mgms. of $CO_2$ per 10 gms. of leaves

| Before injection | | | After injection | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Hours | 3–6 | 6–9 | 9–12 | 12–15 | 15–18 | 18–21 | 21–24 | 24–27 | 27–30 |
| Treatment | | | | | | | | | |
| Air ... | 30·9 | 26·1 | 24·0 | 22·0 | 21·4 | 20·2 | 20·4 | 20·2 | 20·2 |
| Water ... | 30·8 | 25·6 | Injected here | 25·8 | 21·0 | 20·8 | 20·0 | 20·8 | 19·7 |
| 3% ... | 30·1 | 26·6 | | 29·6 | 25·2 | 23·6 | 22·7 | 22·4 | 21·5 |
| 5% ... | 30·4 | 25·1 | | 32·2 | 26·6 | 23·0 | 21·1 | 20·0 | 20·0 |

It will be seen from Tables I and II that in both the kinds of leaves, sooner or later, normal air respiration comes to a steady level phase and that injection with distilled water causes a short-lived enhancement of the respiratory rate in the case of *Mangifera indica*. If however the carbondioxide output of *Allium tuberosum* leaves after injection with distilled water be compared to that before injection, it will be found that the acceleration is almost nil. It is quite possible that the stimulus had spent itself at the time carbondioxide estimation, after water-injection, was begun, as the preliminary output of carbondioxide after each injection was neglected; and thus the respiratory rate was, possibly, measured on its downward trend after the short-lived stimulation.

The weight of alcohol entering the leaves has been shown in Table III.

TABLE III

| Alcohol injected | Mgms. of alcohol entering the leaves (per 10 gms.) |
|---|---|
| *M. indica* | |
| 2% | 45·8 |
| 5% | 130·3 |
| 8% | 202·2 |
| 10% | 244·9 |
| 15% | 331·8 |
| *A. tuberosum* | |
| 3% | 97·2 |
| 5% | 169·8 |

It has already been seen that water-injection accelerates the respiratory rate. Therefore, as aquous solutions of alcohol were used for injection, the acceleration due to water alone should be subtracted from the acceleration produced by a solution of alcohol in water in order to derive the acceleration due to alcohol only. And, as in each experiment, water-injected leaves were used as controls, the acceleration due to alcohol alone can be found out in this way for every dilution of alcohol worked with. The data obtained in this way (from the first carbondioxide estimations after the injections) have been graphically represented, as also the amounts of alcohol entering the leaves, in Fig. 1 (*Mangifera indica*). In the case of 10% alcohol, the second carbondioxide values after injections have been taken into consideration.

As with *Eugenia* leaves,[1] the acceleration of the respiratory rate increases with increasing concentration of alcohol. A concentration beyond a 10% solution, however, brings about a fall. In the case of *Allium* leaves also increasing concentration and amount of alcohol bring about a corresponding rise in carbondioxide output (Table IV). In this case the data for higher percentages of alcohol are, however, not available and therefore the acceleration produced by higher concentrations cannot be deduced.

TABLE IV

| Alcohol injected | Amount of alcohol taken in per 10 gms. of leaves | Increase in $CO_2$ output per 10 gms. of leaves |
|---|---|---|
| 3% | 97·2 mgms. | 3·1 mgms. |
| 5% | 169·8 „ | 6·4 „ |

In order to facilitate comparison, the curves representing the effects of different alcoholic solutions have been shown in the same figures (Figs. 2 and 3 for *Mangifera*

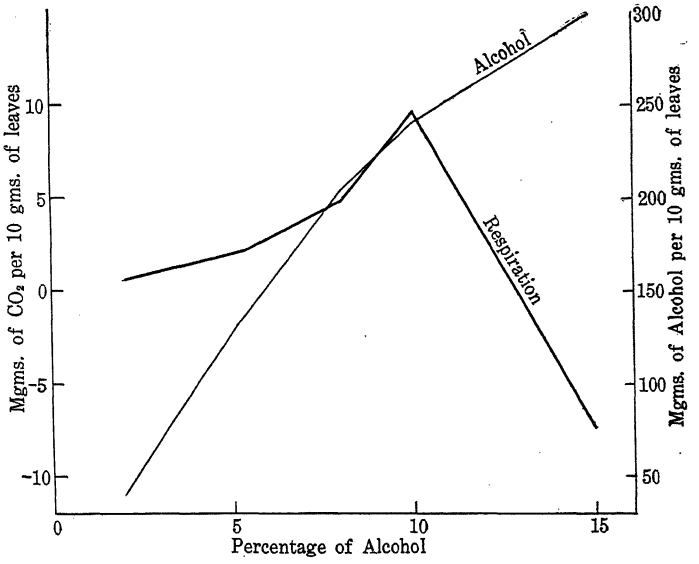


Fig. 1

The amount of alcohol entering the leaves and the respiratory rate (*Mangifera indica*)

*indica* and *Allium tuberosum* respectively). It will be seen that the acceleration increases with the increasing concentration of alcohol and also that the stimulation becomes feebler with the passage of time.

The different curves have been produced backwards in order to obtain carbondioxide values at the zero hour of the application of the stimulus, *i.e.*, at

zero hour after the injections were made. The experimental and derived values obtained in this way of water-injected leaves have been compared in Table V.

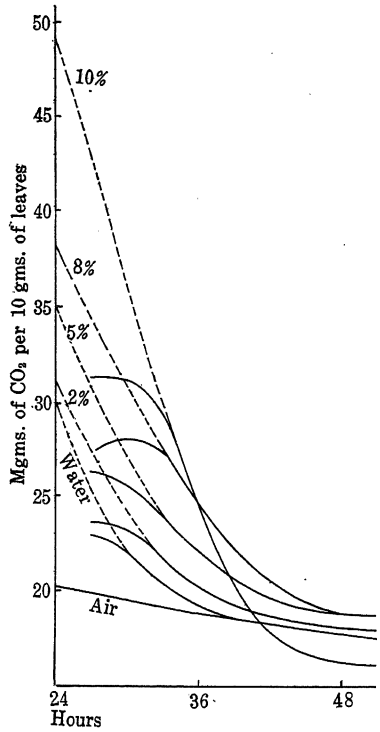


Fig. 2
The effect of different percentages of alcohol on *Mangifera* leaves

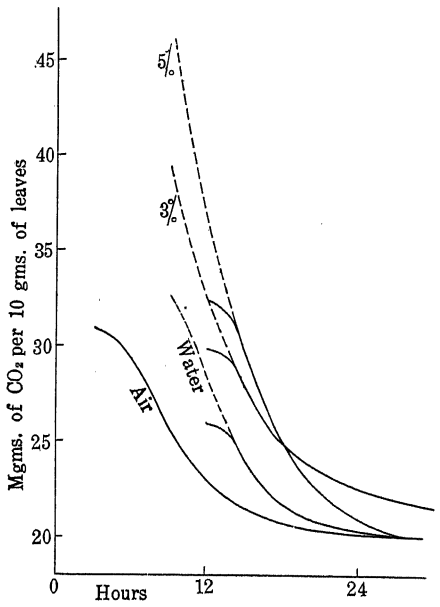


Fig. 3
The effect of different percentages of alcohol on *Allium* leaves

TABLE V

| $CO_2$ of normally respiring leaves | $CO_2$ of leaves injected with water (Experimental) | $CO_2$ of leaves injected with water (Derived) | Acceleration by water | |
|---|---|---|---|---|
| | | | Experimental value | Derived value |
| *M. indica* 20·4 mgms. | 23·0 mgms. | 29·8 mgms. | 2·6 mgms. | 9·4 mgms. |
| *A. tuberosum* 22·0 mgms. | 25·8 mgms. | 32·5 mgms. | 3·8 mgms. | 10·5 mgms. |

The first carbondioxide values after alcohol-injection and those derived by producing the curves backwards are compared in Table VI. It will be seen that the derived values stand higher; this probably indicates a higher acceleration of the respiratory rate at the zero hour or soon after the application of the stimulus, the acceleration becoming feebler with the progress of time.

TABLE VI

| Alcohol injected | $CO_2$ of leaves injected with water (Experimental) | $CO_2$ of leaves injected with alcohol (Experimental) | $CO_2$ of leaves injected with water (Derived) | $CO_2$ of leaves injected with alcohol (Derived) | Acceleration by alcohol | |
|---|---|---|---|---|---|---|
| | | | | | Experimental value | Derived value |
| *M. indica* | | | | | | |
| 2 % ... | 23·0 mgms. | 23·5 mgms. | 29·8 mgms | 31·0 mgms. | ·5 mgms. | 1·2 mgms. |
| 5 % ... | „ „ | 26·2 „ | „ „ | 34·8 „ | 3·2 „ | 5·0 „ |
| 8 % ... | „ „ | 27·2 „ | „ „ | 38·1 „ | 4·2 „ | 8·3 „ |
| 10 % ... | „ „ | 31·2 „ | „ „ | 49·4 „ | 8·2 „ | 19·6 „ |
| *A. tuberosum* | | | | | | |
| 3 % ... | 25·8 „ | 29·6 „ | 32·5 „ | 39·2 „ | 3·8 „ | 6·7 „ |
| 5 % ... | „ „ | 32·2 „ | „ „ | 46·0 „ | 6·4 „ | 13·5 „ |

The percentage increase of carbondioxide output due to alcohol-injection over the normal respiratory rates is shown in Table VII.

TABLE VII

| Alcohol injected | Percentage increase obtained experimentally | Precentage increase from derived values |
|---|---|---|
| *M. indica* | | |
| 2% | 2·5 | 5·9 |
| 5% | 15·7 | 24·5 |
| 8% | 20·6 | 40·7 |
| 10% | 42·0 | 96·1 |
| *A. tuberosum* | | |
| 3% | 17·3 | 30·5 |
| 5% | 29·1 | 61·4 |

It has been pointed out before that the acceleration of carbondioxide production by alcohol decreases with time. This has been shown graphically in Fig. 4 for *Mangifera* leaves from which it can be deduced that 5% alcohol maintains an enhanced respiratory rate for a much longer interval of time than 10% alcohol. In the case of *Allium* leaves also, the data given in Table VIII show clearly that carbondioxide production falls with time after stimulation by alcohol.

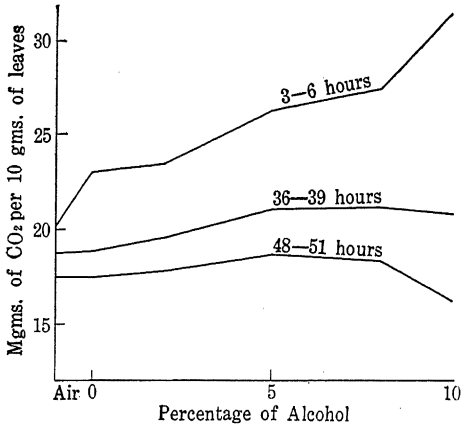


Fig. 4

Effect of alcohol as related to time (*Mangifera indica*)

TABLE VIII

| Alcohol injected | Acceleration over normal respiration | |
|---|---|---|
| | 3—6 hours after injection | 12—15 hours after injection |
| 3 % | 7·6 mgms. | 2·3 mgms. |
| 5 % | 10·2 „ | ·7 mgms. |

The time during which carbondioxide was estimated after injection was 24 hours in the case of *Mangifera indica* leaves. The total amount of carbondioxide evolved during this period by different alcohol-injected leaves have been graphically represented in Fig. 5. From this it will be apparent that 8% alcohol produces the greatest acceleration when a longer interval of time (24 hours) is considered.

As explained in the previous paper[1], the carbondioxide values for normal respiration have been derived from those of water-injected leaves by neglecting the short-lived stimulation produced by water-injection. Thus carbondioxide values for uninjected leaves for the equivalent period, during which the respiratory rates

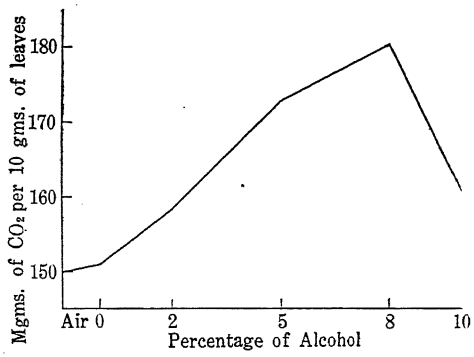


Fig. 5
Total carbondioxide for 24 hours (*Mangifera indica*)

of alcohol-injected leaves were measured, have been obtained. The ratios of total carbondioxide produced by the experimental and control sets before and after the injections have been calculated and compared in Table IX.

TABLE IX

| Alcohol injected | Before injection | | | After injection | | |
|---|---|---|---|---|---|---|
| | $CO_2$ values of control set in mgms. | $CO_2$ values of experimental set in mgms. | Ratio $\frac{CO_2 \text{ experimental}}{CO_2 \text{ control}}$ | $CO_2$ values of control set in mgms. | $CO_2$ values of alcohol set in mgms. | Ratio $\frac{CO_2 \text{ alcohol}}{CO_2 \text{ control}}$ |
| *M. indica* | | | | | | |
| 2 % .. | 179·0 | 180·4 | 1·00 | 147·6 | 158·6 | 1·08 |
| 5 % ... | 186·1 | 188·9 | 1·02 | 150·2 | 173·0 | 1·15 |
| 8 % ... | 189·3 | 189·9 | 1·00 | 148·7 | 180·6 | 1·22 |
| 10 % ... | 186·5 | 185·1 | ·99 | 150·8 | 161·3 | 1·07 |
| 15 % ... | 181·2 | 180·8 | ·99 | 145·7 | 108·3 | ·73 |
| *A. tuberosum* | | | | | | |
| 3 % .. | 56·0 | 56·7 | 1·01 | 42·9 | 54·8 | 1·27 |
| 5 % ... | 56·7 | 55·5 | ·98 | 46·2 | 58·8 | 1·29 |

The different values of $\frac{CO_2 \text{ experimental}}{CO_2 \text{ control}}$ of the above table have been reduced to unity and the corresponding values of $\frac{CO_2 \text{ alcohol}}{CO_2 \text{ control}}$ have been calculated. Thus all the values of $\frac{CO_2 \text{ alcohol}}{CO_2 \text{ control}}$ for the respective leaves become directly comparable (Table X).

TABLE X

| Alcohol injected | Before injection | After injection |
|---|---|---|
| *M. indica* | | |
| 2 % ... ... | 1 | 1·08 |
| 5 % ... ... | 1 | 1·13 |
| 8 % ... ... | 1 | 1·22 |
| 10 % ... ... | 1 | 1·08 |
| 15 % ... ... | 1 | ·74 |
| *A. tuberosum* ... | | |
| 3 % ... ... | 1 | 1·26 |
| 5 % ... ... | 1 | 1·27 |

It is thus evident that the respiratory rate increases with increasing concentration of alcohol in the case of *M. indica*; the limit, however, is reached at 8 % alcohol beyond which carbondioxide production progressively decreases with increasing concentration of alcohol. Such a limit for the leaves of *A. tuberosum* is not possible to fix, as higher percentages of alcohol were not worked with in this case The results seem to be in general agreement with those obtained with the leaves of *Eugenia jambolana* reported in a previous paper.

The author thanks Dr. S. Ranjan for his kind help and guidance.

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# ON THE ANATOMY OF SOME OF THE ASCLEPIADACEÆ

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## SUMMARY

1. Rubiaceous type of stomatal apparatus occurs in most of the plants investigated. Some possess secondary division walls in the subsidiary cells parallel to the pore, while others do not. *Stapelia variegata* Linn., however, exhibits the well-known Tradescantia type.

2. Oxalate of lime occurs in the form of solitary as well as clustered crystals.

3. The hairy covering consists of simple and uniseriate hairs, rarely unicellular hairs (*Stephanotis*). Glandular hairs have been observed in *Sarcostemma*.

4. The petiole contains a single, bicollateral and arc-shaped bundle in *Calotropis*, but it is accompanied by one or more smaller bundles at each and in *Leptadenia*, *Stephanotis*, *Ceropegia*, *Cryptostegia*, *Hemidesmus* and *Pergularia*.

5. Occurrence of laticiferous tubes, superficial development of cork, intra-xylary phloem in cell-groups or in a continuous ring at the margin of the pith, collenchyma in varying amount and assimilatory parenchyma in the primary cortex, and stone-cells in the same region are amongst the other prominent anatomical features.

## INTRODUCTION

This investigation was taken up because the information on the anatomy of the Asclepiadaceæ is rather meagre. It was first proposed to study only the stomatal apparatus, because it is not dealt with in detail by the previous authors. Sabnis (1) describes the stomata of the species he studied, but makes no mention of the type to which they belong. Later on, it was found that the plants under investigation revealed other interesting anatomical features also. Hence the comparative anatomy of the petiole, stem and leaf has been studied. The following species have been investigated: *Caralluma attenuata* Wight., *Leptadenia reticulata* W. & A., *Stapelia grandiflora* Mass., *S. variegata Linn*, *Pergularia odorratissima* W., *Stephanotis floribunda* Brongn., *Sarcostemma brevistigma* W. & A., *Ceropegia spiralis* W., *Calotropis gigantea* Br., *Hemidesmus indicus* R. Br. and *Cryptostegia grandiflora* Br. All these have been recorded by one of the authors (Sayeedud-Din, 2, 3 & 4) either as growing wild or cultivated in Hyderabad.

## Stomatal Apparatus

The stomatal apparatus has been found to belong mostly to the Rubiaceous type with certain variations. This type of stomatal apparatus without secondary division walls in the subsidiary cells is met with in *Pergularia*, *Cryptostegia* and *Hemidesmus indicus*, but in the last mentioned species one subsidiary cell is larger than the other. The Rubiaceous type of stomatal apparatus with secondary division walls in the subsidiary cells parallel to the pore is noticed in *Caralluma*, *Leptadenia*, *Stephanotis*, *Ceropegia*, *Calotropis* and *Stapelia grandiflora*. Stomata surrounded by several ordinary epidermal cells occur in the epidermis of the stem of *Sarcostemma*. *Stapelia variegata*, however, exhibits the well-known Tradescantia type of stomatal apparatus, being surrounded by four subsidiary cells. Jako (Solereder—5) describes a stomatal apparatus resembling the Tradescantia type in *Stapelia* sp. The authors' observations tally with those of Jako, and do not conform to Vesque's findings (Solereder—5). The stomata are deeply sunk in the epidermis.

Leaf—Slight striations in the cuticle are observed in *Leptadenia* and *Calotropis;* and well-striated cuticle occurs in *Hemidesmus* and *Cryptostegia*. Enlargement of the cells on the upper side of the leaf is noticed in *Leptadenia*, *Ceropegia*, *Calotropis* and *Cryptostegia*. This seems to be a device for water storage, as is suspected from the fact that the last three species which grow wild in Hyderabad inhabit dry situations.

Oxalate of Lime occurs in the form of solitary crystals in the stem and leaf of *Calotropis*, *Caralluma*, and *Stapelia* (both the species); and in the form of clustered crystals in *Stephanotis*, *Sarcostemma* (Fig. 14) and *Cryptostegia*. Solitary as well as clustered crystals occur in the stem and leaf of *Leptadenia*, *Pergularia* and *Hemidesmus*.

Laticiferous tubes occur in *Sarcostemma*, *Ceropegia*, *Calotropis*, *Stephanotis*, *Cryptostegia* and *Leptadenia*.

The hairy covering (Figs. 7—12) consists of uniseriate trichomes *(Leptadenia*, *Pergularia* and *Calotropis)*, and unicellular pear-shaped hairs *(Stephanotis)*, as well as 2-celled hairs *(Stapelia variegata)*. Uniseriate clothing hairs as well as glandular hairs occur in *Sarcostemma*.

## Structure of the Petiole

The petiole contains a single bicollateral and arc-shaped vascular bundle accompained by one small vascular bundle at each end in *Leptadenia*, *Stephanotis*, *Ceropegia* and *Cryptostegia*. In *Pergularia* the single arc-shaped bicollateral vascular bundle is accompanied at each end by one, two or three small bundles. The petiole of *Hemidesmus* contains a single bicollateral and arc-shaped vascular bundle accompanied by two small bundles at one end and one small bundle at the

other end. The petiole of *Calotropis*, however, contains only a single bicollateral arc-shaped vascular bundle. Cells with dark contents have been observed in the primary cortex as well as a few in the pith of *Pergularia*. Stone-cells are present in the primary cortex in *Pergularia* and *Ceropegia* (Fig. 13).

## Structure of the Stem

A characteristic feature is the presence of intra-xylary phloem. It occurs either in cell-groups or in a continuous ring at the margin of the pith The primary cortex contains collenchyma in varying amount in *Pergularia*, *Stephanotis*, *Hemidesmus* and *Leptadenia*. Assimilatory parenchyma resembling spongy tissue is found in the primary cortex of *Sarcostemma*. In the same plant stone-cells occur in the primary cortex as well as internally to the pericycle, and sclerenchymatous fibres are met with in the primary cortex (Fig. 14). In the primary cortex as well as in the pith of *Pergularia*, cells with gum-like substance occur in a broken ring. In the same plant, abundant development of vessels is found at four points, and in *Sarcostemma* on two opposite sides. Superficial development of cork has been observed in many of these plants.

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## Explanation of Plates

Fig. 1. Leaf-epidermis.—*Cryptostegia grandiflora* R. Br.

Fig. 2. Leaf-epidermis.—*Hemidesmus indicus* R. Br.

To illustrate Rubiaceous type of stomatal apparatus without secondary division walls in the subsidiary cells.

Fig. 3. Stem-epidermis.—*Caralluma attenuata* W.

Fig. 4. Leaf-epidermis.—*Leptadenia reticulata* W. & A.

To illustrate Rubiaceous type of stomatal apparatus with secondary division walls in the subsidiary cells parallel to the pore.

Fig. 5. Stem-epidermis.—*Sarcostemma brevistigma* W. & A. (Stomata surrounded by several ordinary epidermal cells.)

„ 6. Stem-epidermis.—*Stapelia variegata* Linn. (Tradescantia type of stomatal apparatus.)

(all x 500).

„ 7. Uniseriate trichome.—*Leptadenia reticulata* W. & A.

„ 8 Pear-shaped hair on petiole.—*Stephanotis floribunda* Brongn.

„ 9. Two-celled trichome.—*Stapelia variegata* Linn.

„ 10. Ordinary hair on stem.—*Sarcostemma brevistigma* W. & A.

„ 11. Glandular hair on stem.—*Sarcostemma brevistigma* W. & A.

„ 12. Hair on corolla.—*Ceropegia spiralis* W.

(all x 120)

„ 13. T. S. Petiole.—*Ceropegias piralis* W.
s. c, stone cells; v. b, single arc-shaped vascular bundle.

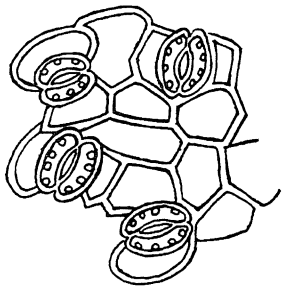
(x 120)

„ 14. T. S. Stem.—*Sarcostemma brevitsigma* W. & A.
c, clustered crystals of Calcium Oxalate; chl., assimilatory parenchymatous cells; scl, sclerenchymatous cells; s. c, stone cells.

(x 120)

PLATE I

M. SAYEEDUD-DIN & M. R. SUXENA—*The Asclepiadaceae.*



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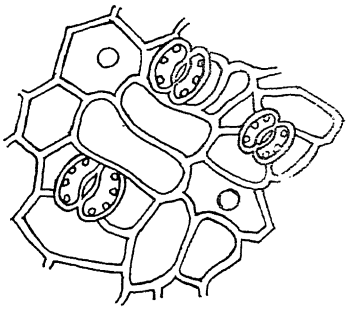

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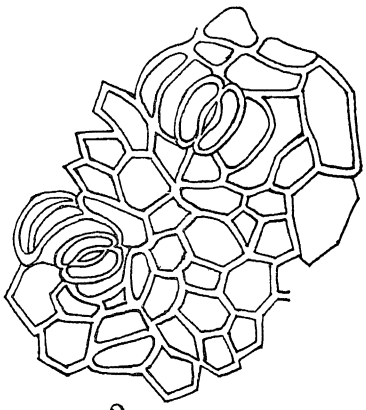

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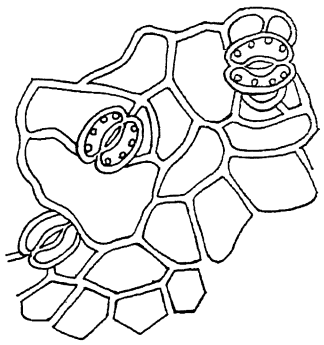

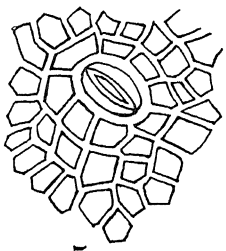

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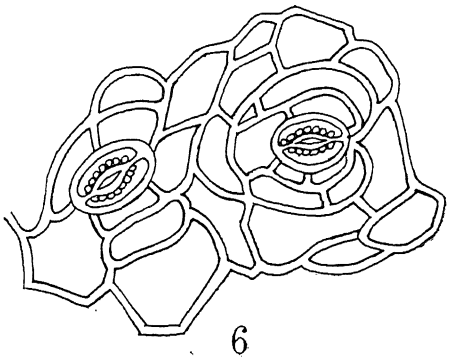

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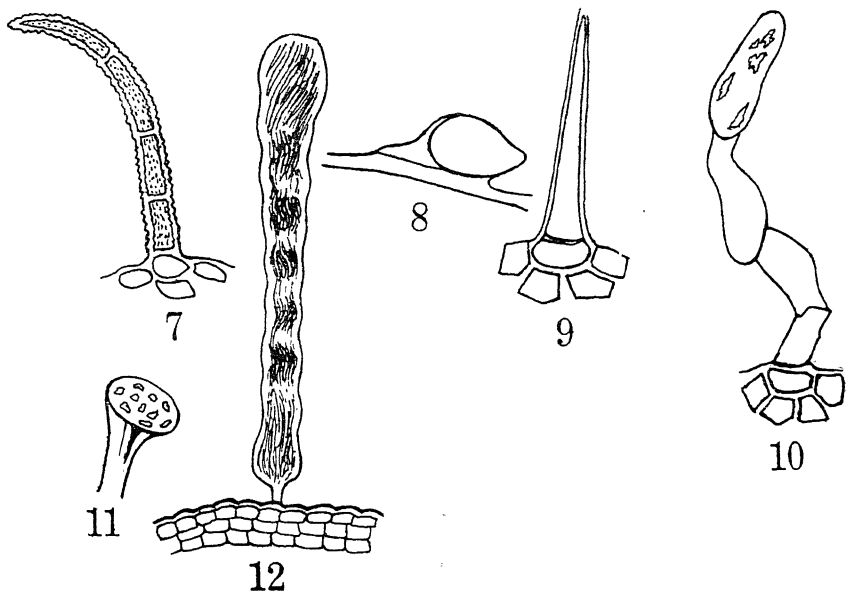
PLATE II

M. SAYEEDUD-DIN & M. R. SUXENA—*The Asclepiadaceae.*



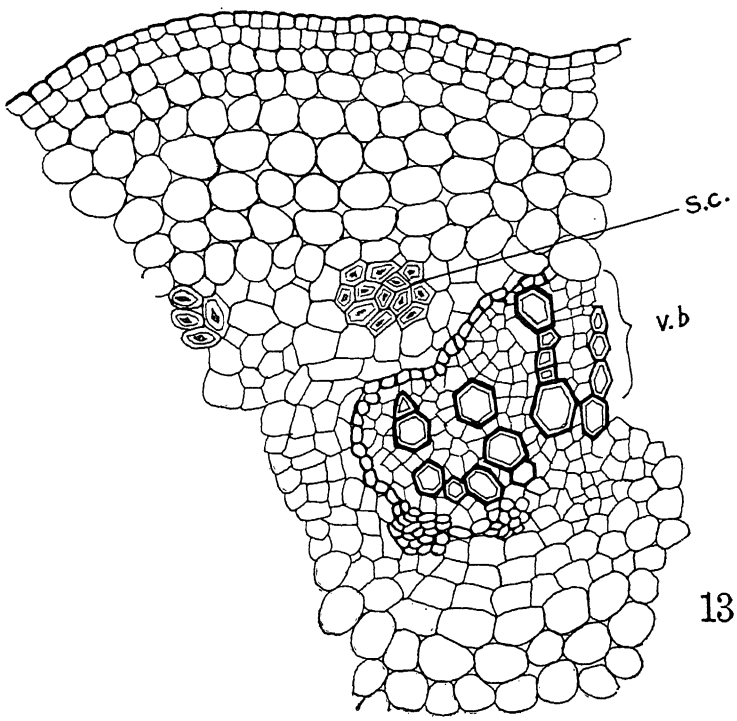
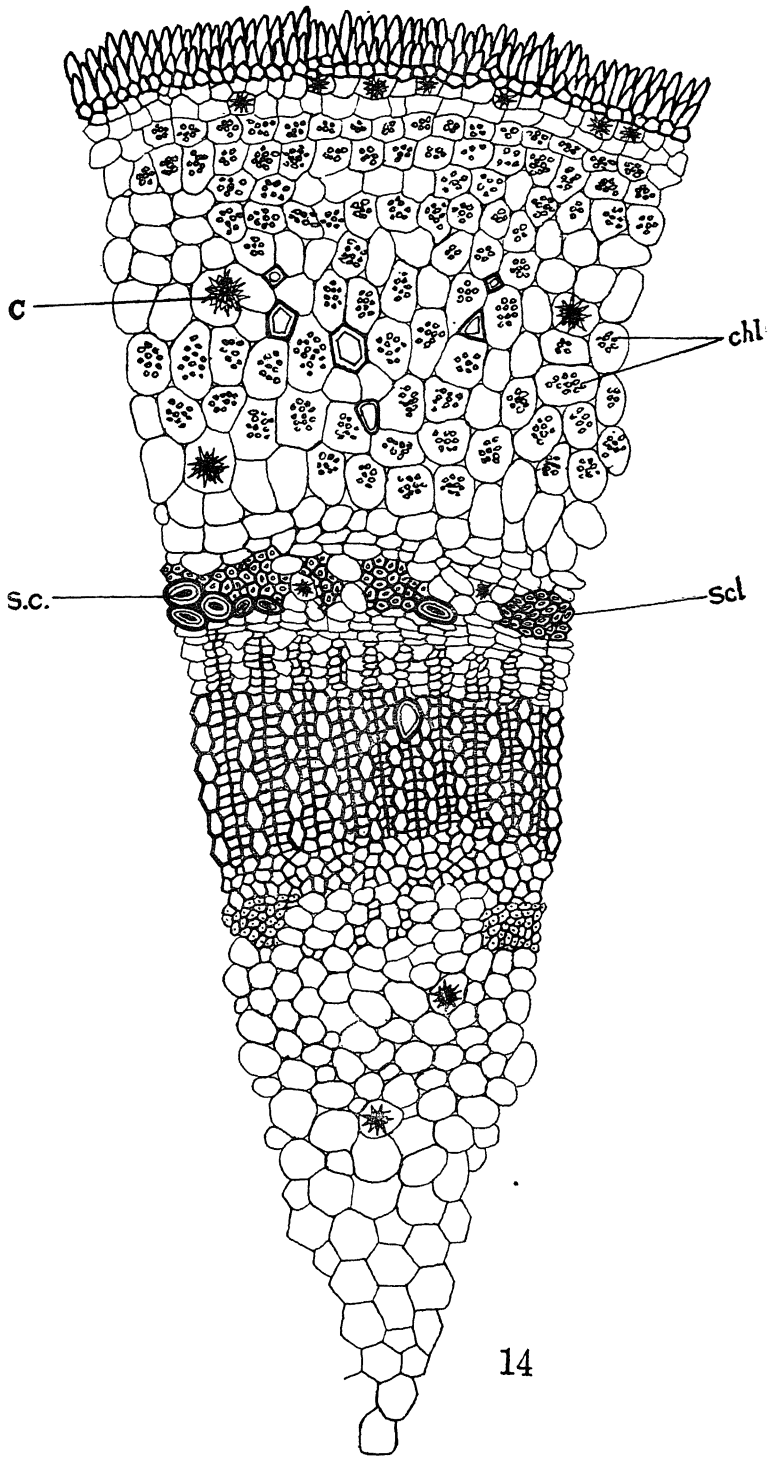



PLATE III

M. SAYEEDUD-DIN & M. R. SUXENA—*The Asclepiadaceae.*



# ON *HÆMOPIS INDICUS* N. SP. A NEW ARHYNCHOBDELLID CARNIVOROUS LEECH FROM KASHMIR

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(Received on April 1, 1940)

SUMMARY

In 1936 specimens of this leech were collected from pools and water channels in Pahlgam, Kashmir. The discovery of this leech in Kashmir is the first record of the genus *Hæmopis* from India. It is a carnivorous and cannibalistic form. The crop is packed with earthworms and leeches swallowed entire.

The alimentary canal of *Haemopis* differs from that of the ordinary blood-sucking leeches. The jaws are very weak, bear two rows of teeth (distichodont), the pharynx is long and muscular extending up to segment IX. The thin-walled crop is a slender, *straight* tube extending up to somite XVIII. There are no paired metameric cæca of the crop as are characteristic of the blood-sucking leeches. A pair of simple tubular cæca which arise from the posterior end of the crop extend up to segment XXII. The crop is followed by stomach, intestine and rectum.

The genitalia and other morphological details are in agreement with those of *Hæmopis sanguisuga* (Linnæus).

## Introduction

In 1936 Prof. K. N. Bahl brought two specimens of a leech from Pahlgam (Kashmir), and kindly handed them over to me for identification and study. Next year on my visit to that place I collected a large number of specimens of the same leech and kept them alive to study their habits, and also made a few dissections which I fixed and preserved for a detailed examination of the internal anatomy. One of these specimens was sent for identification to Prof. J. P. Moore of the University of Pennsylvania, who referred it to the genus *Hæmopis*. The present species is an Arhynchobdellid leech belonging to the family Hirudidæ, sub-family Hirudinæ. This sub-family is divided by Blanchard into two tribes—DISTICHODONTA and MONOSTICHODONTA, according to whether they possess two rows or one row of teeth on their jaws. The tribe DISTICHODONTA includes three genera: *Hæmopis*, *Myxobdella*, and *Whitmania*; while the tribe MONOSTICHODONTA includes several genera: *Dinobdella*, *Hirudo*, *Limnatis*, *Hirudinaria*, and *Pœcilobdella*. Of the three Distichodont genera only two, i.e., *Myxobdella*, and *Whitmania*, have been recorded from India by Moore in the Fauna of British India (1927). Two species of the genus *Hæmopis* Savigny, from India were described by Kaburaki[5] in the Records of the Indian Museum 1921, but both these were found

by Moore to be synonyms of Indian species belonging to other genera, and he, therefore, concluded that the genus *Hæmopis* was not known from India. About the genus *Hæmopis*, Moore[7] writes, "No true *Hæmopis* is now known to occur in India. Several species have been reported but all have proved to be Monostichodont".

In 1886 Whitman [13] described *Hæmopis acranulata*, under the name *Leptostoma acranulatum* from China. In 1930, Moore[8] gave a fairly detailed account of *Hæmopis gracilis* from China.

The discovery of this leech from Kashmir is the first record of the genus *Hæmopis* from India. It differs in many respects from the known species, and there is enough justification for regarding this as a new species. This being the first Indian *Hæmopis* known to science, it is named as *Hæmopis indicus* N. Sp.

The specimens were narcotised in weak alcohol and fixed in 90% alcohol or corrosive sublimate. The dissections, made in normal salt solution, were fixed in Bouin's fluid and preserved in 75% alcohol.

The work was carried out in the Department of Zoology, University of Lucknow. I am indebted to Prof. K. N. Bahl for handing over to me the specimens of this leech collected by him and for finding time to read through and correct the manuscript of this paper. My best thanks are due to Prof. Moore for the determination of the genus and to Dr. Baini Prashad for the loan of necessary literature from the Library of the Zoological Survey of India.

## Locality and Habitat

The specimens were collected from Pahlgam, Kashmir. These leeches are commonly known as burrowing leeches and inhabit swamps and shallow waters and are never found in larger and deeper waters. They are found in large numbers in shallow water concealed beneath stones and pieces of wood, to the undersurfaces of which they attach themselves. At some places they were found burrowing in soft mud near the sides of water channels. To a certain extent they are amphibious since they remain partly out of water and sometimes completely exposed to air. They even make short nocturnal visits on land, in search of food, not far away from water.

## Diagnosis and Colouration

In living condition the body is exceptionally soft and flabby; the integument is perfectly smooth, there being neither papillæ nor tubercles to roughen the surface. The skin always remains moist and slimy on account of an enormous secretion of mucus. A mature specimen, moderately extended, measures 150 to 180 mm. in length and 12 to 18 mm. in width; while *Hæmopis* (*Leptostoma*) *acranulatum* Whitman, measures 120 to 150 mm. when extended and 70 to 80 mm. when at rest, and *H. gracilis* Moore, measures about 67 mm. The body is thick, for the greater part of its length, gently tapering at the anterior and posterior

extremities. The ventral surface is more or less flat and the dorsal is broadly arched, while annulation is very distinct throughout except on the lip. The posterior sucker is small and weak.

The body is of a dark colour in the living condition, without any definite trace of markings. The ground colour on the dorsal surface is olive or olivaceous brown. Some are very dark brown or even almost black on account of a very dense or nearly solid pigmented reticulum on the dorsal surface. The ventral surface is comparatively lighter, and sometimes marked by a few scattered flecks of dark brown pigment. There are no spots and stripes visible in the living condition and the margins also are not differently coloured. On preservation in alcohol for a few days, the pigment fades away, the reticulum becomes lighter, and a careful examination reveals a dense pigmented reticulum on the paramedian fields, which gradually thins out towards the sides. There is then seen a light brown stripe running along the whole length of the body in the mid-dorsal line. The colour patterns of *H. acranulatum*, and *H. gracilis* closely resemble each other, but they differ from that found in the present species.

## Segmentation

The limits of somites are determined on the basis of sensory annuli or the annuli bearing the segmental receptors. The segmental receptors, which are usually small and unelevated, look rather obscure and difficult to spot out in fixed specimens on account of contraction of the body, but in a fresh specimen they are easily seen even without the aid of a hand lens owing to the dark pigmented background. The annulus bearing the segmental receptors is regarded the first annulus of each segment (Whitman).[12] According to this method of counting:

somites I, and II, are uniannulate;

III, XXIV, XXV and XXVI, biannulate,

IV, V, VI, and XXIII triannulate; and

segments from VII to XXII, are quinquannulate.

The total number of annuli is 102. In *H. acranulatum* and *H. gracilis* there are 104 and even 105 annuli.

The body, inclusive of the posterior sucker, is composed of 33 somites (fig. 1) which are grouped into the following regions:

(*i*) The *cephalic* region is composed of a prostomial lobe and somites I to V. It is characterized by the inclusion of the anterior sucker, mouth, jaws and eyes, and by the absence of the nephridial pores. The five pairs of eyes are arranged, as usual, on the dorsal surface of somites I to V: the first three pairs lie on contiguous annuli, while the 4th is separated from the 3rd by one, and the 5th from the 4th by two annuli. The first pair of eyes is the largest and all the five pairs form an

arch on the head region. The eyes are not so easily discernible in the living as they are in fixed specimens.

(*ii*) The *preclitellar* region consists of *three* somites, VI, VII, and VIII. Segment VI is triannulate and the remaining two are completely quinquannulate. All the three segments possess nephridial pores at their ventral caudal ends.

(*iii*) The *clitellar region,* like other Rhynchobdellidæ is formed of *three* complete somites IX, X, and XI. There is no permanent clitellum, as is found in earthworms, but during the breeding season it becomes fairly prominent on account of the thickened layer of clitellar glands. Whitman could not determine the limits of the clitellum in *L. acranulatum*, and Moore had no specimens of *H. gracilis* with definitely developed clitellum. In a few large specimens of the present species the clitellum is seen to be very well developed, extending over the *three* somites (15 annuli) mentioned above. Although the clitellar region differs in colour and texture and is more or less swollen and smoother than the remaining segments, it seldom obscures the annulation. The genital apertures occur on the mid-ventral line of somites X and XI, the male generative aperture in somite X, and the female five annuli behind it in somite XI.

A pair of nephridial pores is present in each of these three segments.

(*iv*) The *middle* region is the largest in the body and consists of *eleven* complete somites, (XII to XXII). All these segments possess nephridia, and the posterior limit of this region extends to the last or the 17th pair of nephridial apertures.

(*v*) The *caudal* region is short and consists of *four* (XXIII to XXVI) incomplete somites out of which segment XXIII is triannulate and the remaining three are biannulate. Segment XXVI bears the anal aperture on its dorsal surface, the last three somites of this region serving as a peduncle for the posterior sucker.

(*vi*) The *posterior sucker* is composed of *seven* segments. All the seven annuli bear segmental receptors, and each annulus represents a segment.

## The External Apertures

The *mouth* opening is placed in the centre of the funnel-like oral-chamber—the anterior sucker. The *anus* is of moderate size and is situated on the dorsal surface of the root of the posterior sucker, in the constriction separating the posterior sucker from the body in somite XXVI. The *apertures of the nephridia* consist of *seventeen pairs* of minute paired openings on the ventral surface of the body, each pair lying on the hindermost annulus of each somite from segments VI to XXII, just in front of the groove separating it from the succeeding segment. The *male* and the *female* generative apertures, as described above, are situated on segments X and XI respectively. They are median and ventral in position,

the two being separated from each other by 5 annuli. The male genital pore is in somite X, a2/a3 in the groove between annuli 30-31, and the female pore in somite XI, a2/a3 between annuli 35-36. A filiform penis, 10-11 mm. in length protrudes out of the male generative aperture in several specimens. In *H. gracilis* it is 1 to 3 mm. in length.

## Alimentary Canal

The alimentary canal of *Hæmopis* (fig. 2) runs as a straight tube from the mouth to the anus It consists of (*i*) the *pre-oral chamber*, and (*ii*) the *buccal cavity*, (*iii*) the *pharynx*, (*iv*) the *œsophagus*, (*v*) the *crop*, (*vi*) the *stomach*, (*vii*) the *intestine*, and (*viii*) the *rectum*

The *pre-oral chamber* (figs 1B, and 2B) is a cup-shaped depression on the ventral aspect of the anterior sucker. The prostomial lobe and the first four segments of the head region form the roof of the oral chamber, while the circular rim of the sucker forms its outer boundary. (The oral chamber is well developed in blood-sucking leeches as it helps attachment on the prey and the sucking of its blood). At the base of the pre-oral chamber lies the *mouth* which is guarded by a thin, wide velum. The *velum* is formed of three triangular muscular folds which project from the walls of the oral-chamber and meet together to give a tri-radiate appearance to the mouth. It is well developed in the blood-sucking leeches, where it forms an almost complete partition between the pre-oral chamber in front and the buccal-cavity behind The mouth leads into the *buccal cavity* which is a very short chamber lying just behind the velum. This chamber accommodates the free anterior marginal folds of the pharynx, and its mucous membrane presents three small crypts, in each of which is embedded a pad-like small compressed jaw (fig. 2D). Of the three jaws, the median is dorsal while the other two are ventro-lateral in position. *Hæmopis* being a carnivorous leech, its jaws are very small and so vestigeal as to escape observation altogether. The jaws are really formed from the termination of the pharyngeal ridges from which they are scarcely differentiated. They are devoid of proper denticles (teeth), and are beset instead with two rows of blunt irregular, thin, cuticular plates, each series consisting of 15 to 20 small plates. In the two lateral jaws the plates are still further reduced in size and number. These plates are separate, partly detached and discrete at some places, while at others several of them are confluent. They are light brown in colour and are much reduced in size at the ends. The buccal cavity leads into the *pharynx* which is a slender fusiform muscular sac extending from somites VI to VIII. Its inner lining is raised into six main longitudinal folds or ridges which are well seen in a transverse section through the middle region of the pharynx, (fig. 2C). Of these folds one is dorsal, one ventral and the remaining two pairs are lateral in position. The two lateral pairs of folds are larger than the median dorsal and the ventrals. There are six smaller ridges alternating with the main ridges and all the

twelve ridges terminate in the three jaws. The pharynx and its ridges are lined internally by a thin layer of cuticle. (In *H. gracilis* the dorsal and the ventral median folds are larger than the two lateral pairs.) The muscle-layers that form the wall of the pharynx are thin and weak. There is an *outer longitudinal layer*, a *middle circular layer* and again an *inner longitudinal layer* of muscles. Of the three layers the middle layer of muscles is comparatively better developed than the other two. There is another set of muscles associated with the pharynx, in which the muscle-fibres extend from the wall of the pharynx to the body-wall ; these are the *radial muscles.* All these muscles produce the sucking action which helps these animals in securing their food. The *salivary glands* (fig. 2E) are masses of unicellular pyriform glands, covering and surrounding the wall of the pharynx. They lie scattered in the space between the radial muscles of the pharynx and the body-wall. These glands are not so well developed in this species as they are in blood-sucking ones. Each cell is a gland by itself, and is produced into a long stalk or ductule. The ductules run forward in bundles along the wall of the pharynx to enter each jaw, in which they occupy an axial position. The papillæ on the flanks of the jaw, on which the salivary glands in the blood-sucking leeches open, are absent in this form, and the salivary ducts open instead into the groove (fig. 2D) placed between the two rows of the plates. The pharynx is followed by a slightly constricted region the *œsophagus*, which continues into the crop. The *crop* (fig. 2A) is a straight thin-walled tube extending from somite IX to the middle of somite XVIII. It shows no trace of metameric division and this feature is characteristic of carnivorous leeches, to which *Hæmopis* belongs. In segment XVIII there arise laterally from the caudal end of the crop, a pair of simple tubular thin-walled cæca which extend backward up to somite XXII. In *H. acranulatum* similar cæca are present, while in *H. gracilis* they are totally absent. The crop is capable of great distension and serves as a store-house for the swallowed food. It continues behind into the *stomach* (fig. 2A) which is a broad thick-walled U-shaped chamber placed in segment XVIII. The two horns of the U project anteriorly as two large pouches, and the belly of the U receives the crop at its concave anterior face and gives out the intestine at its convex posterior end. The wall of the stomach is produced internally into transverse folds which anastomose with one another. In *H. gracilis* the stomach is a simple straight tube. The stomach continues into the *intestine* which is a straight tube showing slight constrictions on both the sides. It extends from somites XIX to XXI. Its inner lining (fig. 2F) presents numerous folds resembling villi, that increase the absorptive surface of the intestine. The wall of the intestine is thinner than that of the stomach and is supplied with numerous blood-capillaries. The intestine continues into the *rectum*, which extends as a simple thin-walled tube from segment XXII to the anus, situated on the dorsal surface of segment XXVI. Absence of villi marks the beginning of the rectum.

## Food

*Hæmopis indicus* is definitely a carnivorous and even a cannibalistic form. The reduced jaws and teeth are incapable of making cutaneous cuts. There is a wide velum, and the alimentary canal is not designed for suction of blood. It feeds on earthworms, leeches of its own species, and small leeches of other genera and species. Dissections of both fresh and fixed specimens showed the crop packed with earthworms and leeches. Large leeches were observed actually to pounce upon the smaller ones and swallow them entire. I have seen leeches and earthworms of an inconveniently large size being swallowed by *Hæmopis*. They are captured by their narrow anterior ends and are drawn in, apparently by *suction*, an action produced by the contraction of the pharyngeal muscles. The mouth which is very mobile becomes much distended during the process of swallowing. *Hæmopis indicus* seems to have a special liking for *Erpobdella octoculata*, [2] a leech which is very commonly found in the locality. At the time of narcotisation a well-fed *Hæmopis* vomits out its crop contents and as many as 6 to 12 leeches and earthworms have actually been seen thrown out through the mouth. In *H. acranulatum*, though the food is not recorded the structure of the alimentary canal appears to be of a carnivorous type, and about *H. gracilis* Moore writes that it is clearly not a sanguivorous leech but a predaceous one that subsists on water-worms, insect-larvae and probably other small animals.

## The Reproductive Organs

*Hæmopis*, like other leeches, is hermaphrodite, each individual possessing both the male and the female generative organs. But the worms are not self-impregnating; cross-fertilization is effected during the copulation of two worms and insemination is reciprocal. The general arrangement of the various parts, though characteristic of the genus, exhibits certain peculiarities in this species.

*(a)* The *male generative organs*—The male generative organs (fig. 3) consist of (*i*) the *testes*, (*ii*) the *vasa efferentia*, (*iii*) the *vasa deferentia*, (*iv*) the *epididymes*, (*v*) the *ejaculatory bulbs*, (*vi*) the *ejaculatory ducts* and (*vii*) the *atrium*.

There are *ten pairs* of *testis-sacs* which are disposed in conformity with the segments from somites XII to XXI, and are arranged in pairs on each side of the nerve cord, each pair lying in the centre of the somite in which it occurs. Each testis-sac is a turgid spherical body full of seminal fluid. From the posterior surface of each testis-sac runs outward a short sinuous duct, the *vas efferens*, which connects the sac with the vas deferens of its own side. The two *vasa deferentia* are a pair of longitudinal tubes that lie on the outer sides of the series of the testis-sacs. They run forward in a sinuous course along the ventral body-wall, parallel to the nerve cord, about mid-way between the testis-sacs and the vesicles (bladders) of the nephridia. Both the vasa deferentia

are covered throughout the testicular region by a thick lobulated layer of unicellular glands. Anterior to the testes, in segments XI and X, they gradually lose their glandular covering, become more slender, and in the middle of somite X, in front of the 7th nerve ganglion, the vasa deferentia bend outwards, become abruptly enlarged and intricately convoluted, to form more or less tubular masses, the *vesicula seminales* (the epididymes). From the outer region of each epididymis emerges a glistening fusiform tube the *ejaculatory bulb*. It has a satiny lustre and is placed on the outer side of the epididymis lying more or less parallel to it in somite X. The anterior end of each ejaculatory bulb narrows down to a slender firm white tube the *ejaculatory duct*, the right or the left duct passing beneath the ventral nerve cord. It has muscular walls and a narrow lumen throughout. The ejaculatory ducts of both the sides bend inward for a short distance and in segment X, near the 6th nerve ganglion, open into a central body the atrium. The *atrium* is a greatly developed and highly muscular U-shaped, tubular organ, the two limbs of which lie in a dorso-ventral position mostly in somites X and XI and sometimes extend even in somite XII. The dorsal limb has at its free anterior end the bulbous prostatic thickening into which the ejaculatory ducts open. The bulbous part has a thick covering of several layers of unicellular glands, the *prostate glands*. Several ducts from these gland-cells open into the lumen of the bulb. The curved part and the entire ventral limb of the atrium together form the *penis sac*. The anterior terminal end of the ventral limb pierces the body-wall and opens to the exterior in segment X a2/a3, just beneath and ventral to the prostatic bulb. Within this is a coiled filamentous tubular *penis*, which when extended is seen as a conspicuous organ protruding through the male generative aperture (fig. 3).

(*b*) The *female generative organs*—The female generative organs (fig. 3) are developed harmoniously with the male organs They are simpler than those of the male and are in the form of a U tube. The two limbs, like the atrial part of the male genital organs, are placed in a dorso-ventral position, the dorsal limb being smaller in diameter than the ventral limb. The dorsal part is made up of (*i*) a pair of *ovaries* enclosed in the ovisacs, (*ii*) the *oviducts* and (*iii*) the *common oviduct*; while the ventral limb consists of (*iv*) the *vagina* only.

The *ovaries* are a pair of minute filamentous bodies which are enclosed in the ovisacs, situated in segment XI, just behind the 7th nerve ganglion. From the base of each ovisac arises a slender *oviduct*, the right or the left oviduct passing beneath the ventral nerve cord. The two oviducts unite into a *common oviduct* in the centre of segment XI. The place of union of the two oviducts is embedded in a thick layer of unicellular *albumen glands*. Each gland-cell has a broad glandular base and a thin drawn-out duct, a number of which pass through the wall of the common oviduct to open into its lumen. The narrow common oviduct runs back to the tail end of segment XII and enters the extreme summit of the vagina,

which is a tubular pouch with muscular walls and a fairly wide lumen. The main chamber of the vagina is the *vaginal cæcum*, while the narrow anterior terminal end in segment XI, is known as the *vaginal stalk*, which opens to the exterior by a small orifice situated in the groove between annuli 2/3 of segment XI.

The genital organs of *H. acranulatum* Whitman (fig. 4) differ from *H. indicus* (fig. 3) in several respects :

1. The number of *testes* is not recorded.
2. The *epididymes* and the *ejaculatory ducts* (the ejaculatory bulbs) are placed more or less in one and the same line. The terminal part of the *seminal duct* (the ejaculatory duct) is very long and runs back along the dorsal side of the *penial pouch* (the penis sac) to enter the pouch near its hind end
3. The *prostatic bulb* is very small and insignificant, and it is situated at the hind end of the penial pouch.
4. The *ovaries* instead of showing the general arrangement characteristic of the genus, exhibit certain peculiarities. They do not lie near the anterior end of the vagina and like the prostatic bulb have shifted their position at the caudal end of the vagina.
5. The *common oviduct* part, covered by the albumen glands, is more compact.
6. The *vagina* is fairly long and is not very plainly differentiated into saccular and tubular parts.

In *H. gracilis* Moore the description of the genital organs is given without a proper representation of the different parts in the sketch. The number of testes recorded is *seven* pairs, and the remaining structures in the genital organs resemble those of *H. acranulatum* Whitman.

Moore [8] writes, "This species (*H. gracilis*) resembles *H. acranulatum* Whitman and differs from typical *Hæmopis* in having the oviduct continued straight cauded of the vagina instead of being doubled forward beneath it. This removes the ovisacs from the typical position in XIII to XVI or XV".

*Hæmopis indicus*, described in this paper, is very similar to the typical *Hæmopis* and its genital organs resemble in most of the details with those of *H. sanguisuga* (Linnaeus) figured by Scriban and Autrum [11] (fig. 296,) after Brandes, in Kükenthal Handbuch der Zoologie, Hirudinea.

ZOOLOGICAL POSITION AND DISTINGUISHING CHARACTERS OF : *Hæmopis indicus* N. Sp.

*Order.*—Hirudinea.

*Sub-order.*—Arhynchobdella.

*Family.*—Hirudidæ.

Ten-eyed, jawed leeches. They are from medium to very large size. Posterior sucker is very constant It includes aquatic, burrowing, and

amphibious forms. They are blood-suckers as well as predaceous and carnivorous. Complete somite is quinquannulate. Clitellum present. Colouration variable. Seventeen pairs of nephridia. The digestive and reproductive organs present considerable variation. They lay cocoons.

*Sub-family.*—Hirudinæ.

There is a great variety of forms. Most members of this sub-family live in swamps and are burrowers rather than inhabitants of larger and deeper waters. Some take solid carnivorous diet, most of them have large, papillated jaws, and are exclusively sanguivorous.

*Tribe.*—Distichodonta.

Jaws small and weak; *teeth mostly in two irregular* rows, few blunt, coarse, often vestigial or thin cuticular plates or absent. Chiefly predaceous. The distichodonts are in many respects the most primitive and in their predatory habits (feeding on worms, insect larvae and other smaller invertebrates, varied by an occasional meal of blood), their relatively simple digestive tracts, weak and often edentulous or even vestigial jaws, they stand nearest to the Erpobdellidae.

Their reproductive organs are complex.

*Genus.—Hæmopis.*

Integument peculiarly soft. Posterior sucker small, velum tumid, projecting into the oral cavity in the form of papillae. Jaws very small, distichodont (two rows of teeth) with very few imperfect cuticular plates incapable of making cutaneous cuts. Predaceous, alimentary canal simple, crop without metameric cæca.

*Species.—Hæmopis indicus* N. Sp.

(*i*) *Size*—80 to 150 mm. in length, 12 to 18 mm. in breadth.

(*ii*) *Integument* perfectly smooth, devoid of tubercles and papillae.

(*iii*) *Colouration* very dark owing to a dense dark brown or black reticulum.

(*iv*) Body composed of thirty-three segments which are divided in the following *six* regions:—

*Cephalic* I-V, *preclitellar* VI-VIII, *clitellar* IX-XI, *middle* XII-XXII, *caudal* XXIII-XXVI, and *posterior sucker* of seven segments.

(*v*) The male generative aperture is in segment X a2/a3, and the female aperture five annuli behind it in segment XI a2/a3. Seventeen pairs of nephridiopores from segments VI to XXII.

(*vi*) The *alimentary canal* is very simple. Three jaws are very small and weak, teeth in the form of blunt, irregular plates in two rows, 14 to 15 plates in each row. Pharynx is a muscular bulb, its inner wall is raised

into ridges. Crop is straight and simple without any metameric caeca. A single pair of long, slender, straight caeca arise from crop in segment XVIII, which run back up to segment XXII. Intestine is simple.

(*vii*) *Food.* The jaws are incapable of making cutaneous cuts. This form is predaceous, carnivorous, and even cannibalistic, feeding on leeches, earthworms, and insect-larvae

(*viii*) Reproductive system.

*Ten pairs of testes* from somites XII to XXI. Epididymes in segment X; *ejaculatory bulbs* arise from the epididymes, and *ejaculatory ducts* open into a large tubular *atrium. Prostatic bulb* covers the 6th ganglion, and *penis sac* is in segments X and XI.

A pair of *ovisacs* enclosing the ovaries in segment XI.

The *common oviduct* runs back and opens into a large tubular *vagina.*

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## Explanation of Plates

### Fig 1. *Hæmopis indicus* N. Sp.

A. dorsal; B. ventral aspect.

*an.* anus; *as.* anterior sucker; *e. 1.* first and *e 5.* fifth pair of eyes; *g. p.* male generative aperture; *g p.* female generative aperture *n. p. 1.* first and *n. p. 17.* seventeenth pair of nephridiopores; *p.* prostomium; *pen.* penis; *p. s.* posterior sucker; *s. r. o.* segmental receptor organ; I-XXVI. the body segments.

### Fig. 2. *Hæmopis indicus* N. Sp.

A. *Alimentary Canal*; the pharynx opened out to show the pharyngeal ridges.

*an.* anus; *br.* brain; *ca.* crop cæcum; *c. m.* circular layer of muscles, *cr.* crop; *int.* intestine; *n. c.* nerve collar; *œs.* œsophagus; *ph* pharynx; *p. r* pharyngeal ridges; *rect.* rectum; *r. m.* radial muscles; *sal. g.* salivary glands; *st.* stomach; *v. n. c.* ventral nerve cord; V-XXVI segments

B. *Longitudinal section of the first eight segments.*

*b c.* buccal cavity; *b. r* brain; *cu* cuticle; *j.* jaw; *o. c.* oral chamber; *p.* prostomium; *ph.* pharynx; *r. m.* radial muscles; *sal. g.* salivary glands; *v. n. c.* ventral nerve cord.

C. *Transverse section through the middle region of Pharynx.*

*c. m.* circular layer of muscles; *cu.* cuticle; *ph. l.* pharyngeal lumen: *r. m.*, radial muscles;

D *Jaw.*

*j.* jaw; *j. m* jaw muscles; *s. o.* salivary openings; $t.^1$ and $t^2$, two rows of cuticular teeth.

E. *Salivary Glands.*

*s. d.* Salivary duct; *s. g.* salivary glands.

F. *Longitudinal section of the posterior part of the alimentary canal.*

*cr.* crop; *ca.* cæcum; *i. f.* intestinal folds; *int.* intestine; *rect.* rectum; *st.*, stomach; *s. f.* stomach folds.

### Fig. 3. Reproductive organs of *Hæmopis indicus* N. Sp.

In the male reproductive organs the terminal portion, *i.e.*, the last pair of testis-sacs and atrium are shown, *d. e.* ductus ejaculatoris; *e b.* ejaculatory bulb; *ep.* epididymis; *p. g.* prostate glands; *p b.* prostatic bulb; *p. s* penis sac of atrium; *T. 10.* Tenth pair of testis sac; *v. e.* vas efferens; *v. d.* vas deferens; *v. n. c.* ventral nerve cord.

*The female reproductive organs.*

*al. g.* albumen glands; *c ov. d.* common oviduct; *ov. d.* oviduct; *ov. s*, ovisac; *va. s*, vaginal stalk; *v. c.* vaginal cæcum; *v. n. c.* ventral nerve cord *6-7-8-9.* The nerve ganglia; IX, X. XI, XII segments.

### Fig. 4. Reproductive organs of *Leptostoma* (*Hæmopis*) *acranulatum*, after Whitman.

The male organs open between the 6th and 7th nerve ganglia (counting the subœsophageal as one); the female organ between the 7th and 8th. The ovaries have shifted their position from between the 7th and 8th ganglia to a point just in front of the 12th ganglion. The Vagina and penial pouch are extremely long. *1-2 t.* testes; *v d.*, vas deferens commune; *v. s.* vesicula seminalis (epididymis); *d.* ductus ejaculatorius; *p g.* prostate gland; *p.* penial pouch (sacculus penis); *ov.* ovaries; *al. g.* albumen gland; *c. ov. d.*, common oviduct; *v.*, vagina; *v. n. c.* ventral nerve cord; 7 to 12 nerve ganglia.

PLATE I

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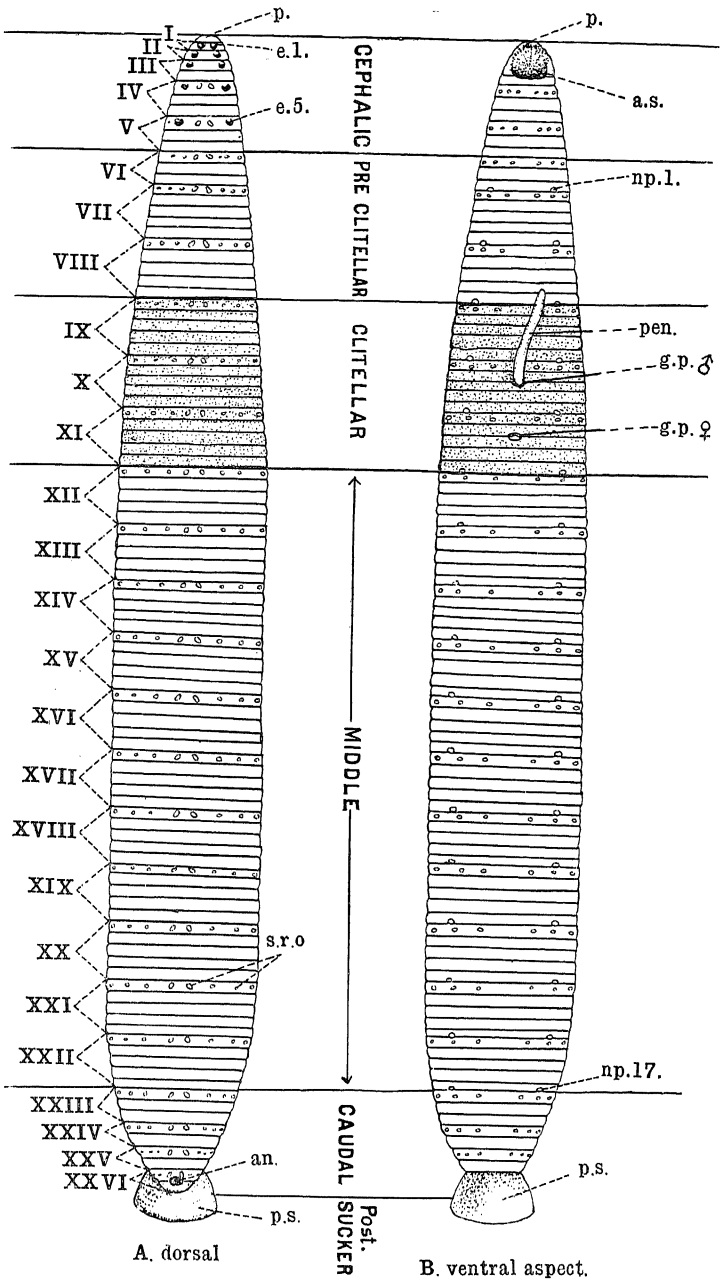
*Hæmopis Indicus* External Characters.




A. dorsal

B. ventral aspect.

PLATE II

*Hæmopis Indicus* Alimentary Canal

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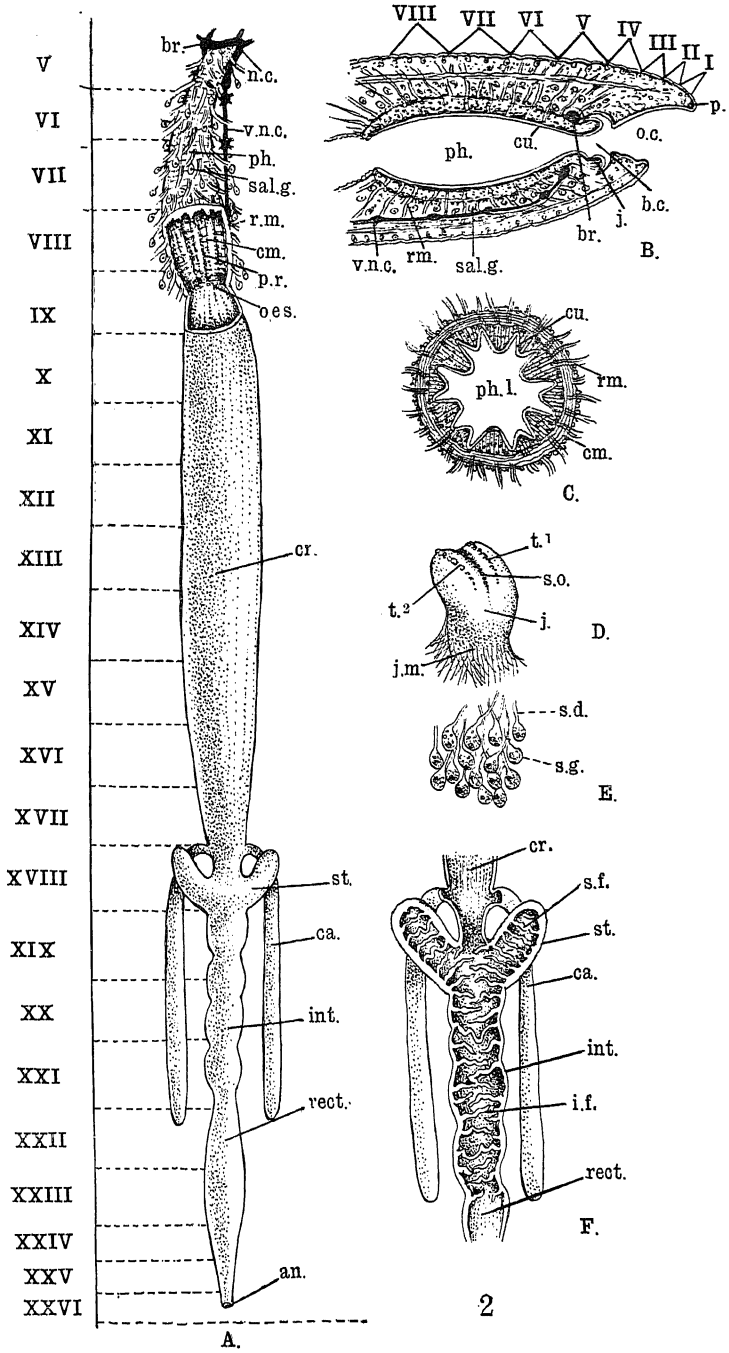

PLATE III

*Hæmopis Indicus* Reproductive organs.

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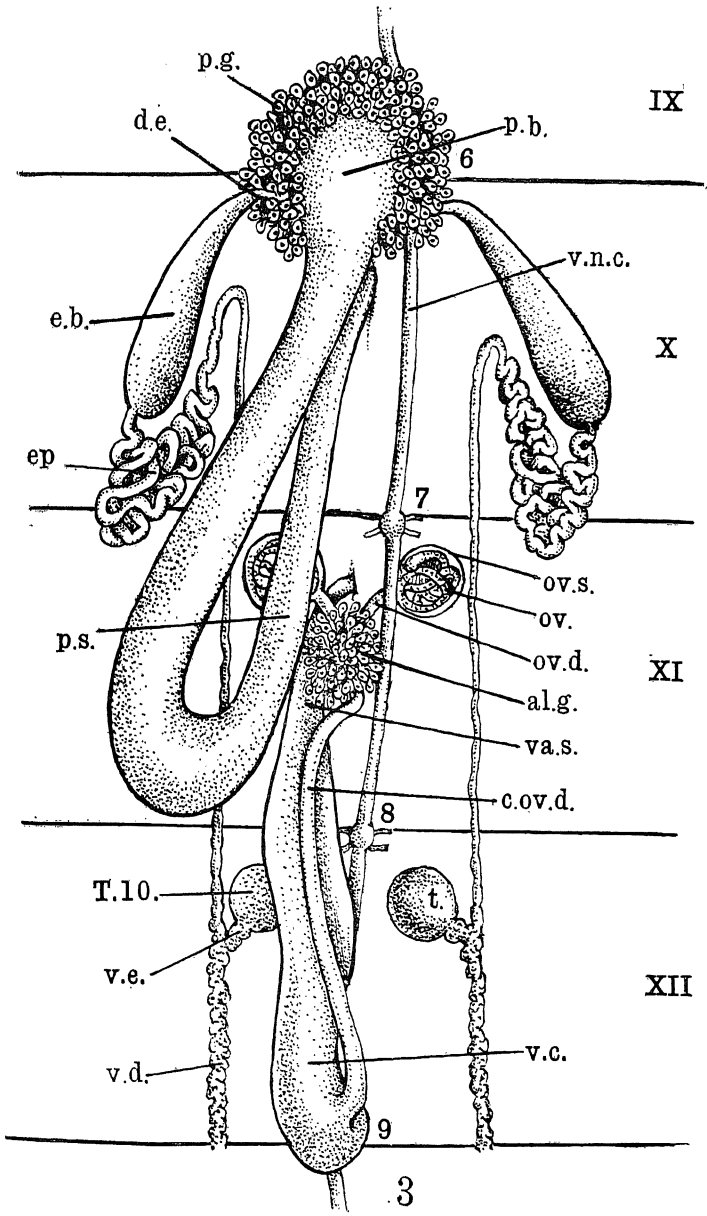

PLATE IV

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*Leptostoma* (Hæmopis) *acranulatum* Reproductive organs